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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The Effect of Increasing Sodium Intake on Sympathetic Nervous System Activity in Normotensive Mice

by

Anita M. Simon

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

Nutrition

FACULTY OF HOME ECONOMICS

EDMONTON, ALBERTA

FALL, 1988

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **The Effect of Increasing Sodium Intake on Sympathetic Nervous System Activity in Normotensive Mice** submitted by Anita M. Simon in partial fulfillment of the requirements for the degree of Master of SCIENCE in Nutrition.

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Date Jan. 23, 1988

Dedication

I thank all who have loved me in their hearts,
With thanks and love from mine.

To My Parents, Dr. and Mrs. John B. Simon,
and
My Brother, Butch.

Thank you for your undivided loving support.

Abstract

The relationships between sodium (Na^+) intake and sympathetic nervous system (SNS) activity were assessed in mice fed 0.05%, 1.00%, or 3.00% Na^+ for varying durations. Acutely, SNS activity assessed as norepinephrine (NE) turnover after synthesis inhibition did not differ between mice fed 0.10% Na^+ or 3.05% Na^+ for 1 day; 0.05% Na^+ or 1.00% Na^+ for 5 days, or 0.05% Na^+ or 3.00% Na^+ for 5 days in brain, kidney, heart or interscapular brown adipose tissue. Subsequently, the effects of increased Na^+ intake (from 0.05% to 3.00% Na^+) on the short-term adaptation pattern of urinary Na^+, creatinine, free NE and free dopamine (DA), subchronic 13 day increases in Na^+ intake on cardiac and renal NE turnover rate and chronic 30 day increases in Na^+ intake on central and peripheral NE turnover in normotensive mice were investigated. Na^+ excretion was greater in mice fed the 3.00% Na^+ diet within 1 day of supplementation and remained elevated throughout the 10 day study. Urinary creatinine was greater in mice fed 3.00% Na^+ compared to those fed 0.05% Na^+ on days 4,6,7 and 9. 24 h urinary free NE excretion expressed in absolute terms, or in relation to creatinine declined over time in mice fed the low Na^+ diet such that free NE was lower in mice fed 0.05% Na^+ diet compared to those fed the 3.00% Na^+ diet on days 2,4 and 6 through 10. Similarly, 24 h free DA excretion decreased in response to the low 0.05% Na^+ diet. To determine the organs at which sympathetic neuron function was being altered in response to feeding a low 0.05% Na^+ diet subchronically, mice were fed diets containing 0.05% or 3.00% Na^+ for 13 days and NE turnover assessed in heart and kidney. Fractional and total NE turnover rates were 57% and 79% lower, respectively, in heart only of mice fed the 0.05% Na^+ diet compared to those fed the 3.00% Na^+ diet. In mice fed chronically 3.00% or 0.05% Na^+ diets for 30 days, NE turnover did not differ in any organ examined. These results suggest that differences in sympathetic activity, produced by alteration in dietary Na^+ within a physiological range are temporal and reflect suppression of noradrenergic activity in response to prolonged consumption of a low Na^+ diet rather than stimulation of sympathetic activity in response to a high Na^+ diet.

Acknowledgements

Special thanks to my supervisor, Dr. J.L. Johnston for her continued support, guidance, and encouragement; to my committee members, Dr. T. Clandinin, Dr. P. Trayhurn, and Dr. G. Baker for their examination of this thesis; and to my friends and colleagues, for their support throughout.

This work was supported by grants from NSERC.

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I. Introduction

The Effect of Increasing Sodium Intake on Sympathetic Nervous System Activity: A Proposal for the Induction of Hypertension

The autonomic nervous system consists of sympathetic and parasympathetic divisions (Figure I-1). The sympathetic nervous system (SNS) plays a major role in the regulation of cardiovascular function and systemic circulation. The vasomotor center of the brain stem transmits impulses downward through the spinal cord and then through the postganglionic sympathetic vasoconstrictor fibers to all or almost all the blood vessels of the body and to the heart (Figure 1-2). Anatomical differences between the sympathetic and parasympathetic divisions of the autonomic nervous system indicate the relative importance of each system on the regulation of circulation. Sympathetic activity affects heart rate, heart contractility and degree of vasoconstriction whereas parasympathetic activity affects cardiac output only. Functional consequences of altered sympathetic activity on systemic circulation in relation to altered sodium (Na^+) intake have been proposed as a mechanism by which dietary Na^+ influences blood pressure.

Epidemiological data indicate that populations with a high Na^+ intake have a high prevalence of hypertension (Scribner 1983; Berglund 1983). The hypothesis that a high Na^+ intake in some way is responsible for the initiation and maintenance of essential hypertension has led to increased research designed to clarify the relationship between dietary Na^+ and blood pressure regulation. One hypothesized link is the relationship between Na^+ intake, Na^+ homeostasis and neural function. This review will survey the reported effects that increasing Na^+ intake has on neural function, specifically SNS activity, and will relate this effect to proposed mechanisms by which Na^+ intake is thought to influence SNS activity.

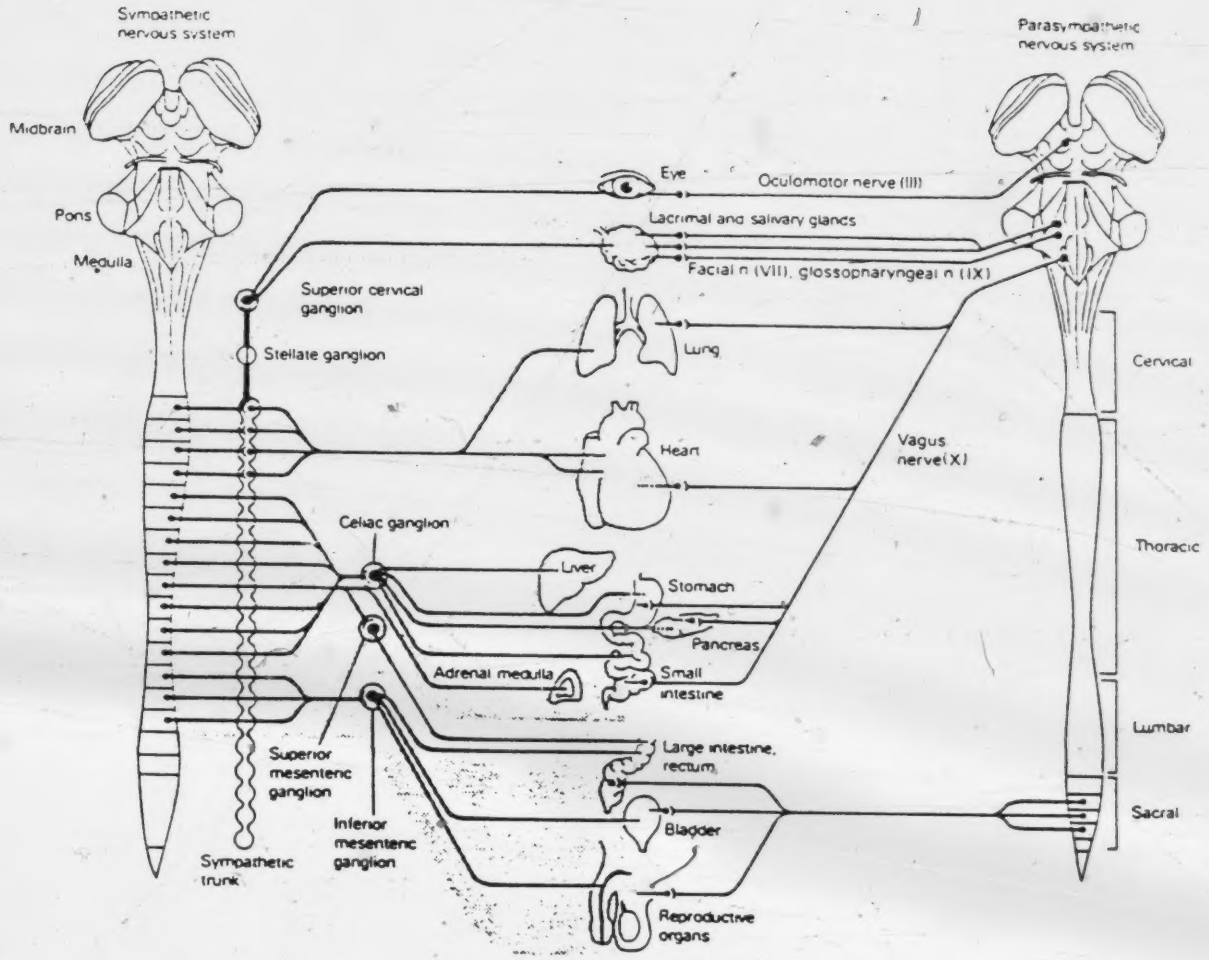

Figure I-1 The autonomic nervous system consists of sympathetic and parasympathetic divisions. The connections of both systems with the hypothalamus and higher brain centers have been omitted in this figure (From Kandel and Schwartz 1985).

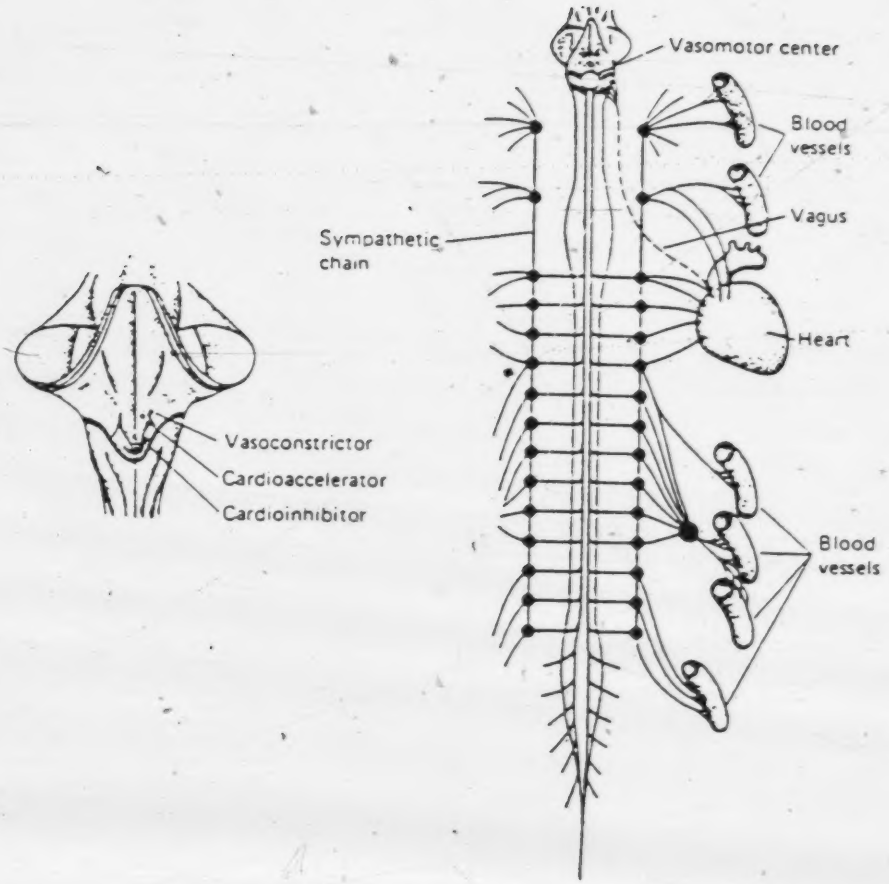

Figure I-2 The vasomotor center in the brain stem and its relationship to the circulatory system through the postganglionic sympathetic nerves and through the vagus nerve to the heart. which is part of the parasympathetic nervous system (From Guyton 1986).

A. Sympathetic Activity and Essential Hypertension

Primary or essential hypertension is not linked to a single etiology. Heredity predisposes an individual to hypertension, but environmental, neurogenic, humoral, and vascular factors also interact and influence blood pressure to various extents (Horan et al. 1985). Most patients with essential hypertension show heightened vascular and cardiac reactions to sympathetic stimuli including norepinephrine (NE) and sympathomimetic amine infusion and increased dietary Na^+ (Goldenberg 1948; Trendelenburg 1965; Parfrey et al. 1981; Skrabal et al. 1984). It may be that enhanced SNS activity, as reflected by elevated plasma NE, is associated with increased salt sensitivity and, consequently, predisposition towards essential hypertension (Campese 1982; Dustan 1985; Skrabal et al. 1984). Salt-sensitivity is based on blood pressure responses to salt-loading whereby humans categorized as 'salt-sensitive' display a rise in mean arterial pressure equal to or greater than 10% after Na^+ intake is increased from low to high levels, from 10 to 200 mmol Na^+/day, respectively. 'Non-salt sensitive' humans show a change in blood pressure less than 10% when given a similar Na^+ load (Campese 1982; Kawasaki et al. 1978; Koolen and Van Brummelen 1984). Animal studies suggest that increased salt-sensitivity may arise from a genetic or acquired deficiency in the kidney's ability to excrete Na^+, or from increased vascular resistance secondary to enhanced SNS activity and concommitant release of the chronotropic pressor agent NE (Dustan 1985; Abboud 1984).

Increased vascular resistance secondary to excessive sympathetic drive has been described by Abboud (1984) to be an important factor in the development of hypertension. In human hypertension, increased sympathetic activity has been recorded from peripheral nerves by a microneurographic technique employing tungsten microelectrodes to record multiunit sympathetic activity in skin and muscle nerves of normotensive and hypertensive subjects (Wallin et al. 1973). Campese et al. (1980) proposed increased sympathetic neuron activity, as reflected by elevated levels of plasma NE, to be a major pathogenic factor in the earlier stages of this disease, whereas increased vascular responsiveness to NE

or other non-neurogenic factors may become major determinants in the maintenance of blood pressure in the later stages of hypertension.

Lesions at specific sites in the central nervous system such as the nucleus tractus solitarius of the medulla have been shown to cause excessive sympathetic activity and sustained hypertension in the rat (Doba and Reis 1974). Furthermore, sympathetic abnormalities have been identified in various models of hypertension. In two genetic models of hypertension including the Dahl-salt-senstive rat and the spontaneously hypertensive rat (SHR), there appears to be facilitated release of NE during sympathetic stimulation (Takeshita and Mark 1978; Collis et al. 1980).

Interventions which reduce sympathetic nerve activity have been shown to reduce or prevent hypertension. Predominant among these is the lesion of the anteroventral portion of the third ventricle in rats which has been shown to correct and also prevent hypertension in a variety of animal models (Brody et al. 1980; Abboud 1984). Neurochemical interventions which interfere with sympathoadrenal discharge or with adrenergic receptors on the effector cell such as the α - adrenergic agonists clonidine or prazosin, will lower blood pressure (de Champlain et al. 1968).

Sodium depletion has been proven to be one of the most effective mechanisms for reducing arterial pressure and appears to exert its effect through a reduction in sympathoadrenal drive (de Champlain 1968; Brosnihan 1981; Mark et al. 1975).

It is therefore possible that a high Na^+ intake is responsible for the development of essential hypertension through its stimulatory effect on SNS activity.

B. Relationships Between Sodium Intake and Peripheral Sympathetic and Dopaminergic Activity

The exact mechanism by which dietary Na^+ affects SNS activity remains to be determined. Changes in dietary Na^+ intake could affect sympathetic neuronal function in several ways. First, Na^+ ingestion influences the haemodynamic state by affecting body fluid volume which in turn may lead to reflex adjustments in the activities of the

sympathetic and dopaminergic systems and the renin-angiotensin aldosterone system (Davis and Freeman 1976; de Champlain 1976). Second, dietary Na^+, by altering extracellular Na^+ concentration, may affect neuronal function indirectly by initially stimulating the release of natriuretic hormone, as proposed by Blaustein (Blaustein and Hamlyn 1983; 1984).

1. Sodium Homeostasis and Sympathetic and Dopaminergic Activity

Na^+ homeostasis and peripheral SNS function are closely related. Evidence has been obtained that the kidney possesses both alpha-and beta-adrenergic receptors and is directly innervated with NE-containing nerve terminals (Schrier 1974; Hall et al. 1974; Vollmer 1984). In the kidney, the adrenergic neurohumoral transmitter NE is primarily an alpha-adrenergic agonist. Increases in renal sympathetic tone, therefore, produce vasoconstriction of the renal vasculature at several sites including the afferent and efferent arterioles as well as the venules (Schrier 1974). With marked adrenergic stimulation, renal vascular resistance increases as a result of vasoconstriction at all of these locations and,subsequently, both glomerular filtration rate and renal blood flow decrease, promoting Na^+ reabsorption with involvement of the macula densa natrioreceptor (Schrier 1974).

NE has also been shown to affect Na^+ balance directly by stimulating beta adrenergic receptors in renal juxtaglomerular cells, thereby increasing renin release. Evidence for this direct sympathoadrenergic action independent of vascular and macula densa receptors comes from Johnson et al. (1971) who showed that electrical stimulation of the renal nerves could increase renin release in dogs with nonfiltering kidney (and therefore inactivated macula densa) and with the vascular receptors inactivated by the vasodilating agent papaverine (Johnson et al. 1971).

Alpha and beta adrenergic receptors in juxtaglomerular cells have opposite influences on renin release. The stimulating influence of the beta receptors appears to be mirrored by a suppressing action of alpha receptors which can be seen both in the intact

animal and in the isolated perfused kidney or in kidney slices (Zanchetti 1985). The SNS, therefore, is believed to play an important role in achieving or maintaining Na^+ homeostasis by altering the functional interrelationships among the macula densa, the juxtaglomerular cells, and the glomerulus. Specifically, noradrenergic activity may alter Na^+ excretion indirectly through alpha adrenergic mechanisms, leading to renal vasoconstriction and subsequently decreased glomerular filtration rate and Na^+ reabsorption, or directly through beta adrenergic mechanisms affecting renin release and, via the renin-angiotensin aldosterone system, increasing Na^+ reabsorption (Johnson et al. 1971; Davis 1971; Kopp and Di Bona 1984).

Evidence for a direct role for neurotransmitters in the regulation of Na^+ balance has involved the study of isolated perfused kidneys and proximal tubules in which infused NE or renal nerve stimulation produced renal vasoconstriction, reduced natriuresis, and increased renin secretion (Morgunov and Baines 1981; Imbs et al. 1984). Renal α-adrenergic blockade has been shown to reverse the antinatriuretic response to renal nerve stimulation (Morgunov and Baines 1981). In contrast, clearance studies indicate that dopamine (DA) promotes natriuresis and is directly related to dietary Na^+ (Alexander et al. 1974; Oates et al. 1979). Intravenous and renal arterial infusions of DA have been shown to increase Na^+ excretion through a direct vasodilating effect on renal vasculature, decreasing peritubular oncotic pressure, filtration fraction and, subsequently, Na^+ reabsorption (Imbs et al. 1984). Dopaminergic receptors 1 and 2 have been characterized and shown to promote vasodilation of renal and mesenteric beds and inhibit presynaptic NE release, respectively. Dopamine has also been shown to hold α-and β-adrenergic receptor activity (Miller 1984).

Further evidence for a natriuretic role for DA and an antinatriuretic one for NE came from studies relating catecholamine excretion and dietary Na^+ intake. A direct correlation between Na^+ intake and urinary DA excretion and an inverse on with NE were noted (Oates et al. 1979). The opposite effects of NE and DA on the renal handling of Na^+ suggest that

both peripheral sympathetic and dopaminergic activity play important roles in the regulation of Na^+ homeostasis. SNS activity, in turn, may be expected to be influenced by dietary Na^+ to effectively maintain Na^+ balance.

The precise mechanism by which dietary Na^+ may influence noradrenergic neuronal function is unknown. De Champlain suggested that the state of Na^+ balance could affect NE disposition in peripheral sympathetic neurons (de Champlain et al. 1968). He proposed that the ability of sympathetic granules to store NE was inversely related to Na^+ intake. This hypothesis was supported by the finding that in deoxycorticosterone acetate (DOCA) salt hypertensive rats, NE concentration in cardiac sympathetic neurons was reduced. In addition, field-stimulated [^{3}H] NE release was increased relative to normotensive controls, indicating an increased rate of firing or decreased ability of the neurons to take up and retain NE. Conversely, when these rats were Na^+ depleted following maintenance on a low Na^+ diet (0.05%Na^+) for three weeks and treatment with a diuretic, cardiac NE content increased (de Champlain and Van Ameringen 1981). Similarly, Cambotti-Kaufman et al. (1984) reported that DOCA-Na^+ hypertensive rats maintained on a low Na^+ diet for one month but without diuretic treatment, had higher NE concentrations in cardiac, renal, and mesenteric arterial tissue than their high Na^+ fed counterparts (Cambotti-Kaufman et al. 1984). Increased peripheral SNS activity following Na^+ restriction provides a mechanism by which hypotension, resulting from decreased blood volume secondary to decreased Na^+ availability for renal reabsorption, can be prevented through intrarenal and extrarenal systemic alpha-adrenergic vasoconstriction.

There is increasing evidence that high Na^+ intakes are also associated with increased SNS activity in humans, as measured by plasma concentration and urinary excretion of catecholamines (Luft et al. 1979; Romoff et al. 1979; Nicholls et al. 1980). For example, Nicholls et al. (1980) showed that when Na^+ intake was increased from 120 to 240 mmol/day, plasma free NE increased approximately 50% in normotensive men. It is possible, therefore, that hypertension associated with high Na^+ intakes may be attributed to

a maladaptive response wherein dietary Na^+ enhances SNS activity which may lead to reduced Na^+ excretion and increased vascular resistance through alpha-adrenergic actions.

2. Natriuretic Hormone Hypertension Hypothesis

A mechanism by which high Na^+ intakes might alter neuronal function is summarized by the "natriuretic hormone hypertension hypothesis" proposed by Blaustein and Hamlyn (Blaustein and Hamlyn 1983; 1984). The hypothesis states that defective, active Na^+ transport out of several cells, including arterial smooth muscle cells and sympathetic neurons, is the underlying cause for essential hypertension. The hypothesis suggests that a high Na^+ intake leads to the release of a hypothalamic natriuretic hormone which then inhibits Na^+-potassium (K^+) pumps in many cells including renal tubular cells, vascular smooth muscle cells and sympathetic neurons (Blaustein and Hamlyn 1983). By inhibiting active Na^+ transport across renal basolateral membranes and out of the cells, the natriuretic hormone promotes natriuresis and inhibits reabsorption so that Na^+ homeostasis may be achieved. In vascular smooth muscle cells, however, inhibition of the Na^+-K^+ ATPase pump would lead to decreased Na^+ -calcium (Ca^{2+}) counter transport since less extracellular Na^+ is available to move down its concentration gradient into the cell and fuel Ca^{2+} counter transport out of the cell. The resultant increase in intracellular Ca^{2+} concentration then leads to increased vascular tone since in vascular smooth muscle intracellular, cytoplasmic Ca^{2+} is the normal trigger for contraction. The Ca^{2+} that triggers activation of vascular smooth muscle cells enters the cytoplasm from two sources: (1) from the extracellular fluid, via voltage- and transmitter-regulated Ca^{2+}-selective channels in the plasma membrane, and (2) from the intracellular stores in the sarcoplasmic reticulum (Blaustein and Hamlyn 1984). NE released from sympathetic nerve terminals or varicosities triggers contraction by promoting Ca^{2+} entry into the cytoplasm from both sources (Blaustein and Hamlyn 1984). Thus, if there is increased release and/or delayed

removal of NE, smooth muscle is activated for a longer than normal period, and blood pressure should increase.

The primary mechanism for terminating the postsynaptic action of NE is by its extensive reuptake by the neurons (Blaustein and Hamlyn 1984). This reuptake is mediated by a Na^+-NE cotransport system that derives its energy from the Na^+ electrochemical gradient across the neuronal plasma membrane (Haddy et al. 1973). Partial inhibition of the Na^+ pumps by circulating natriuretic hormone causes the Na^+ electrochemical gradient to decline primarily as a result of an increase in intracellular Na^+ concentration. As a result, the rate and extent of NE reuptake will be reduced and more NE will remain in the interstitial space, prolonging the activation of vascular smooth muscle effector cells (Haddy and Overbeck 1976).

The normal trigger for the release of catecholamines from adrenergic neurons is an increase in intracellular Ca^{2+} (Blaustein and Hamlyn 1984). Ca^{2+} ion transport across neuronal and plasma membranes depends, in part, upon counterflow exchange of Na^+ for Ca^{2+}. This exchange is dependent on extracellular Na^+ concentration which, in turn, is dependent on ATPase activity and the Na^+-K^+ transmembrane gradient. Therefore, a decline in the Na^+ electrochemical gradient will lead to decreased Ca^{2+} transport out of the cell, which in turn will lead to NE release (Blaustein and Hamlyn 1983; 1984). This Na^+-dependent increase in intracellular Ca^{2+} should enhance spontaneous NE release, as well as the release evoked by nerve stimulation (Blaustein and Hamlyn 1983; 1984; Blaustein 1977). The increase in NE release, and decrease in NE reuptake will increase vasocorstriction beyond that seen with Na^+-K^+ ATPase inhibition by natriuretic hormone and subsequent contraction of arteriolar smooth muscle cells (Blaustein and Hamlyn 1984; Blaustein 1977).

The natriuretic hormone hypertension hypothesis was inspired by previous studies that showed Na^+-K^+ ATPase activity to be suppressed in various cells including arteriolar, venous smooth muscle cells and cardiac cells from animals with low renin hypertension

(Haddy and Overbeck 1976; Iversen 1973). In 1976, Haddy reported that Na^+-K^+ pump activity, as estimated by ouabain-sensitive ^{86}Rb uptake, is suppressed in the arteries and veins of dogs with one model of low renin hypertension (one-kidney). Subsequent studies observed the same changes in ouabain-sensitive ^{86}Rb uptake and Na^+-K^+ ATPase activity in other models of low renin hypertension (one-kidney, one clip: one-kidney, DOCA, salt ; reduced renal mass, salt) but not in two genetic models of hypertension (Haddy and Overbeck 1976). Haddy also showed that suppression of Na^+-K^+ pump activity increased the contractile activity of arterial smooth and cardiac muscle and that ouabain produced a large increase in arterial pressure in the anesthetized dog prepared so that diuresis could not occur.

The investigation of Na^+ transport in human hypertension has relied heavily on cells other than those from the vascular smooth muscle itself, particularly platelets, erythrocytes and leukocytes. Since vessel walls have been shown to undergo structural changes when hypertension is present, it is difficult to be confident that any alterations in composition are not simply a consequence of this transformation. Furthermore, samples of blood vessels from living normotensive and hypertensive patients can rarely be obtained (Hilton et al. 1986). There is, therefore, extensive literature on cellular Na^+ transport of blood cells in human essential hypertension (Hilton et al. 1986; Poston et al. 1981; Ambrosione et al. 1981).

Cytosolic free calcium concentration in platelets has been correlated to systolic or diastolic pressure and was reported to be significantly higher in hypertensive humans than in those of normotensives. Increased cytosolic calcium concentrations were postulated to be secondary to Na^+-K^+ ATPase inhibition in association with hypertension (Sang and Devynck 1987). Similarly, increased diastolic pressure has been associated with increased levels of intracellular Na^+ and with a diminished value for the rate-constant of Na^+ efflux in leukocytes from patients with essential hypertension indicating suppressed ATPase activity (Poston et al. 1981; Ambrosioni et al. 1981; Hilton et al. 1986). It is apparent, however, that these cells can have no direct involvement in the mechanism of hypertension and that

the study of them is of value only to the extent that their transport processes are representative of those that take place in other body tissues.

Karaki et al. (1978) showed that in rabbit aorta, ouabain and K^+-free solution produced a concentration-dependent potentiation of NE contraction that was inhibited by infusion of phentolamine, an alpha-adrenergic blocker. Karaki et al. concluded that ouabain and K^+-free solution affect contraction both indirectly via the release of endogenous catecholamines and directly by acting on vascular smooth muscle. Caution in interpretation of data is warranted here as, although larger blood vessels such as the aorta and its main branches are the most convenient for the assay of cellular Na^+-K^+ transport, they are known to differ both anatomically and functionally from the smaller resistance vessels and may therefore be less appropriate for the study of Na^+ metabolism in hypertension (Hilton et al.1986).

Evidence for the involvement of a circulating Na^+-K^+ pump inhibitor in the genesis of essential hypertension is derived from a study conducted by Hamlyn et al. (1982) in which a highly significant correlation was found between levels of a plasma inhibitor of Na^+-K^+ ATPase activity and mean arterial pressure in age-matched normotensive and hypertensive individuals. Na^+-K^+ ATPase inhibition was significantly greater in the hypertensive group (Skrabal 1984). Moreover, incubating leukocytes from normotensive subjects in serum obtained from humans with essential hypertension was found to cause an impairment in Na^+ transport similar to that found in the leukocytes of the hypertensive group (Hamlyn et al. 1982).

Various laboratories have used plasma, urine, or tissue as potential sources of the Na^+-K^+ ATPase inhibitor (Haupert et al. 1984; Akagawa 1984; Gruber et al. 1980; Haber and Haupert 1987; Flynn and Davis 1985). Most of these efforts are at a preliminary stage. Gruber et al. (1980) partially purified from dog plasma a peptidic substance that inhibited Na^+-K^+ ATPase and whose concentration varied with volume expansion. Because the midbrain has been implicated in the control of circulating Na^+-K^+ ATPase inhibitors, the

brain as a source of the natriuretic hormone has been extensively studied. Brody et al. (1987), seeking a site of control for ATPase activity, demonstrated that lesions in the region of the anteroventral third ventricle of the brain prevented the hypertension of volume expansion. Haupert and colleagues (1984) isolated and partially purified extracts from bovine hypothalamus that were applied to the serosal surface of the toad urinary bladder and produced reversible, nontoxic inhibition of active Na^+ transport across the membrane. Infusion of the hypothalamic inhibitor into isolated perfused rat kidney produced natriuresis. The hypothalamic inhibitor was shown to be a low-molecular-weight nonpeptidic substance that appeared to be hydrophilic. In contrast, partially purified bovine hypothalamic ATPase inhibitor was reported to be peptidic in chemical nature by Akagawa et al. (1984). Recently, peptides extracted from rat atrial tissue were sequenced and found to promote natriuresis and diuresis when injected into rat plasma (Flynn and Davis 1985).

Further investigation is needed to determine the source, chemical nature and physiologial significance of the natriuretic hormone. The relations between ATPase activity, SNS activity, renal and vascular hemodynamics and Na^+ homeostasis remain unclear.

C. The Effect of Increasing Na^+ Intake on SNS Activity

1. Studies of Normotensive Humans

An assessment of human studies on the effect of Na^+ intake on SNS activity reveals results that are contradictory. That is, certain studies on the effect of Na^+ loading indicate increases in sympathetic activity (Berglund 1983; Campese 1982; Dustan 1985; Parfrey et al. 1981; Nicholls et al. 1980) while others indicate decreases (Luft et al. 1979; Romoff et al. 1979; Nicholls et al.1980). For example, the stimulatory affect of Na^+ on SNS activity is illustrated by the study of Nicholls et al. (1980) in which plasma free NE increased significantly when 12 normotensive subjects increased their Na^+ intake from 120 to 240 mmol/day for one week. Conversely, Luft et al. (1979) reported that when Na^+ intake increased from 10 to 300, 600, 800, 1200, or 1500 mmol/day for three days, plasma and urinary free NE decreased in normotensives. One explanation for this contradiction may be the lack of specificity and sensitivity of plasma and urinary measurements. Plasma and urinary NE measurements are limited by their dependence on the rate of removal from circulation, rate of release from sympathetic neurons and by their inability to detect regional differences in SNS activity (Esler et al. 1984; Folkow et al. 1983). Specificity may be improved at least in experimental animals by calculating NE turnover using the rate of disappearance of injected [^{3}H] NE from tissues or the rate of decline in NE concentration after synthesis inhibition by α-methyl-para-tyrosine (de Champlain 1976; Folkow 1976).

Luft et al. (1979) showed that when normotensive humans increased their Na^+ intake from 10 to 300 mmol/day for three days, plasma NE concentration and urinary NE decreased 58% and 72% respectively (Luft et al. 1979). Similarly, Romoff et al. (1979) have shown plasma NE concentration to be highest when Na^+ is restricted at 10 mmol/day and to decrease by 42% when Na^+ intake is increased to 200 mmol/day during a seven day period (Romoff et al. 1979). These results suggest a decrease in noradrenergic activity when Na^+ intake is increased from a level near the daily

requirement to the rather high level of a typical North American diet. In a study conducted by Nicholls et al. (1980), 12 normotensive subjects were given three different levels of Na^+ intake (10, 120, and 240 mmol/day) for one week. Plasma NE concentration was shown to decrease by 64% when Na^+ intake increased from 10 to 120 mmol/day. However, when Na^+ intake was further increased from 120 to 240 mmol/day for the same length of time, plasma NE concentration increased by 53% (Nicholls et al. 1980). Parfrey et al. (1981) showed that plasma NE concentration decreased by 18% when the subject's habitual diet was supplemented for 12 weeks with 100 mmol Na^+ daily (Parfrey et al. 1981). Berglund (1983) found that urinary excretion of NE doubled when Na^+ intake increased from 180 to 340 mmol/day after four weeks. The different dosage and duration of Na^+ supplementation make these studies difficult to compare but lead to the speculation that there may be a time-and dose-dependent sympathetic response to Na^+ intake. However, taken at face value and assuming Na^+ homeostasis had been achieved, the reviewed studies suggest there is not a linear relationship between Na^+ intake and SNS activity. Instead it would appear that a U-shaped relationship exists. At the recommended Na^+ intake of 0.5-0.6 g(9 - 26 mmol)/day (RNI, 1983), sympathetic activity may be increased to compensate for a blood pressure fall induced by the decrease in blood volume (as part of a renal conservation mechanism). SNS activity appears to decline as Na^+ intake is increased to normal intake levels, 2 to 5 g Na^+/day. When Na^+ is increased beyond this range, SNS activity again appears to be elevated.

2. Studies of Hypertensive Humans

A relationship different from the above studies in normotensive individuals between Na^+ intake and SNS activity has been demonstrated in salt-sensitive subjects with essential hypertension. Campese et al. (1982) have shown that hypertensive subjects receiving 200 mmol Na^+/day for three weeks have plasma NE concentrations 84% greater than normotensives consuming a similar Na^+ intake. Parfrey et al. (1981) found patients with

mild essential hypertension to have higher plasma NE concentration than normotensive subjects on similar unregulated diets. These studies suggest that SNS hyperactivity may be a causal or contributing factor to the development of hypertension.

3. Studies of Normotensive Animals

Experiments investigating the effects of increasing dietary Na^+ on noradrenergic activity in normotensive animals provide limited evidence that a negative correlation between Na^+ intake and SNS activity exists. In a study involving normotensive Wistar rats chronically given low Na^+, basal, or high Na^+ diets containing 0.02%, 0.66%, or 6.87% Na^+, respectively for five weeks, Kaufman and Vollmer (1984) found kidney, ventricular, atrial, and arterial tissue NE concentrations and baseline and electrically stimulated plasma NE concentrations were affected by Na^+ intake. Baseline plasma NE concentration was elevated approximately 64% in low Na^+-fed rats as compared to basal but not high Na^+-fed rats. In response to sympathetic stimulation (4 Hz, 50 V for 40 sec), the plasma NE level in low Na^+-fed rats was 69% higher than in basal and 116% higher than in high Na^+-fed rats. NE concentrations measured in the above tissues were consistently higher in rats maintained on the low Na^+ diet as compared to basal and high Na^+-fed rats (Kaufman and Vollmer 1984).

Similarly, Saavedra et al. (1983) reported that a one week increase in dietary Na^+ from 0.18% to 3.2% reduced the activities of tyrosine 3-hydroxylase by 5% and dopamine β-hydroxylase by 18% in Dahl Na^+-resistant rats thereby associating the lower Na^+ diet with enhanced NE synthesis (Saavedra et al. 1983). In contrast, de Champlain et al. (1969) reported increased NE turnover in the heart, intestine, and spleen of normotensive rats when fed a 1% sodium chloride (NaCl) diet for four to six weeks.

In a study conducted by Tanaka et al. (1982), NE turnover rate was estimated from the decline in tissue NE concentration after blockade of NE synthesis by α-methyl-para-tyrosine in normotensive rabbits fed low, normal, or high Na^+ diets containing 0.005%,

0.4%, or 2.46% Na^+ respectively for three weeks. Na^+ intake exerted opposite effects on NE turnover in cardiovascular tissues and brain stem. In the thoracic aorta, mesenteric vein, and left ventricle the rate of NE turnover was directly related to Na^+ intake such that its rate approximately doubled with each increase in Na^+ intake from low (0.005%) to high (2.46%) Na^+ levels. Conversely, in the hypothalamus, midbrain, pons and medulla, NE turnover was inversely related to Na^+ intake and was lower in both 2.46% and 0.4% Na^+-fed rabbits when compared to 0.005% Na^+-fed rabbits (Tanaka et al. 1982). These studies suggest that intraspecies and interspecies differences exist in relation to tissue noradrenergic responses to altered Na^+ intake.

4. Studies of Hypertensive Animals

Winternitz and Oparil (1982) investigated Na^+-neural interactions in the pathogenesis of hypertension in the spontaneously hypertensive rat of the Okamoto strain (SHR). Male SHRs were given diets of either low (0.05%), normal (0.29%) or high (3.4%) Na^+ contents for three weeks, after which plasma NE concentration, urinary NE excretion, and blood pressure were measured. In SHRs receiving the highest Na^+ diet, plasma NE concentration and urine NE excretion were 40% and 77% higher, respectively, than in rats fed the low or normal Na^+ diets. In addition, a highly significant positive correlation was seen between plasma NE concentration and systolic blood pressure (Winternitz and Oparil 1982). These results support the hypothesis that the increase in blood pressure seen with hypertension associated with a high Na^+ intake is in part due to enhanced peripheral sympathetic activity.

de Champlain et al.(1969) reported increased NE turnover assessed by the rate of disappearance of endogenous NE after inhibition of tyrosine hydroxylase by α-methyl-para-tyrosine, in heart, intestine and spleen of control rats and those made hypertensive by DOCA and Na^+ administration. The rats were fed a 1% NaCl diet for four to six weeks. NE turnover rate was greater in all tissues examined in the hypertensive animals

(de Champlain et al. 1969). Similarly, Dietz et al. (1980) found sympathetic activity, as assessed by plasma NE concentration, to increase with Na^+ loading in spontaneously hypertensive rats. In this study, male stroke-prone spontaneously hypertensive (spSH) rats and control Wistar Kyoto (WK) rats were fed either a high Na^+ diet containing 3.5% Na^+ or a standard Na^+ diet of 0.57% Na^+ for 14 days. Plasma NE concentration was 72% higher in the high Na^+-fed spSH rats, compared to the standard Na^+-fed spSH rats (Dietz et al. 1980).

D. Implications

The relationship between Na^+ intake and SNS activity is not fully characterized. A review of both human and animal studies indicate a relationship between dietary Na^+ and sympathetic activity exists. Whether this relationship is U shaped as normotensive human studies suggest, direct and positive as hypertensive animal studies indicate, or negative as normotensive animal studies tend to indicate, has yet to be determined.

Studies in normotensive humans indicate that low Na^+ intakes between 10 and 100 mmol/day are negatively correlated to plasma NE concentration or urinary NE excretion. Conversely, at high Na^+ intakes (200-360 mmol/day), dietary Na^+ and plasma or urinary NE concentration are positively correlated in humans. Although these results suggest that a U-shaped or curvilinear relationship exists between Na^+ intake and SNS activity, the apparent nonlinearity of such a relationship may be due to differences between studies in: (1) duration of Na^+ intake, (2) concentration of Na^+ in diet, and/or (3) method of assessing SNS activity

In contrast, studies in hypertensive animals indicate that a positive correlation may exist between Na^+ intake and SNS activity. Although the mode of assessing SNS activity was generally consistent in these studies and involved the measurement of NE turnover , the effects of Na^+ intake on sympathetic activity cannot be accurately described since duration of Na^+ treatment and dietary Na^+ concentration varied between studies. To fully

characterize the relationship that exists between Na^+ intake and SNS activity, Na^+ concentration and Na^+ treatment duration need to be investigated separately to determine their relative, independent effects on sympathetic activity. A more complete assessment of the dietary Na^+ effect would involve studying the effects of different levels of Na^+ intake on SNS activity, ensuring that the duration of Na^+ treatment and method of SNS assessment be maintained constant. Similarly, to determine how the duration of Na^+ concentration affects sympathetic activity, studies must be designed to alter Na^+ treatment duration while keeping Na^+ intake and methodology for SNS assessment constant. Such studies would then be able to distinguish the effects of Na^+ concentration from Na^+ duration of feeding on sympathetic activity and would fully characterize the time and/or dose dependent course of SNS response to Na^+ intake. Consequently, these studies may also clarify the relative contributions of Na^+ intake and Na^+ concentration towards the genesis of hypertension.

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II. Effects of Acute Increases in Sodium Intake on Central and Peripheral Norepinephrine Turnover in Normotensive Mice

A. Introduction

Several lines of evidence suggest enhanced sympathetic activity may be responsible for the induction or maintenance of hypertension associated with a high Na^+ intake. Epidemiological data indicate that populations with a high Na^+ intake have a high prevalence of hypertension (Scribner 1983; Berglund 1983). Enhanced sympathetic activity, reflected by elevated plasma or urinary NE, may be associated with increased salt sensitivity and, consequently, predisposition towards essential hypertension (Campese 1982; Dustan 1985; Skrabal 1984). Na^+ supplementation has been shown to enhance sympathetic activity in both hypertensive and normotensive humans and animals. It is therefore possible that a high Na^+ intake predisposes an individual toward hypertension through its effect on SNS activity.

Sodium supplementation in experimental animal models indicates that Na^+ intake is directly related to sympathetic activity. Increases in Na^+ intake from 0.05% to 3.4% for three weeks increase plasma NE concentration and urinary NE excretion by 40% and 77%, respectively, in spontaneously hypertensive rats (Winternitz and Oparil 1982). Similarly, Dietz et al. (1980) found plasma NE concentration to increase with Na^+ loading in spontaneously hypertensive rats when Na^+ intake increased from 0.60% to 3.40% for 14 days. Plasma NE measurements, however, are subject to several limitations. One is the dependence of the plasma concentration on plasma clearance of NE. A second is the uncertainty that exists as to the source of the NE, should the plasma level be elevated. Urinary NE measurements also do not indicate the source of altered NE spillover should NE excretion change, and as well assume that a constant fraction of plasma NE is excreted in the urine (Folkow 1982; Elser 1982).

Central and peripheral NE release rate and peripheral NE turnover increase in animal models of hypertension receiving Na^+ supplementation. Rats made hypertensive by deoxycorticosterone acetate (DOCA) and 1% sodium chloride show an increased rate of disappearance of endogenous NE in the heart, intestine, and spleen relative to normotensive controls (de Champlain et al. 1969). Stimulated [^{3}H] NE release from portal vein and anterior hypothalamus have also been shown to be directly related to Na^+ intake in spontaneously hypertensive and Wistar-Kyoto control rats (Meldrum et al. 1982).

Experiments investigating the effects of increasing dietary Na^+ on noradrenergic activity in normotensive animals is limited, providing inconsistent evidence that a negative correlation exists between Na^+ intake and SNS activity. Kaufman and Vollmer (1984) found kidney, ventricular, atrial, and arterial tissue NE concentration decreased when Na^+ intake increased to 0.66% or 6.87% from 0.02% Na^+ for five weeks. Plasma NE levels were also shown to decrease approximately 64% with similar increases in Na^+ intake. Similarly, Tanaka et al. (1982) found NE turnover rate to decrease in the brain stem when dietary Na^+ increased from 0.005% to 0.4% or from 0.4% to 2.46% Na^+ for three weeks. However, opposite effects were reported in cardiovascular tissues, involving the thoracic aorta, mesenteric vein and left ventricle where a direct relationship between Na^+ and NE turnover was observed (Tanaka et al. 1982). Normotensive animals in these experiments were fed at extremes of Na^+ intake (Kaufman et al. 1984; Tanaka et al. 1982). Therefore, the relationship between Na^+ intake within a physiological range and noradrenergic activity is unclear and hence the role of Na^+ intake in the genesis of hypertension is undetermined.

Variations in dietary Na^+ concentration and in treatment duration in normotensive humans and animal studies make it difficult to assess the relationship between Na^+ intake and noradrenergic activity. Consequently, the role of dietary sodium as a single factor in the development of the neurogenic component of hypertension is unclear. Before the role of dietary Na^+ in hypertension can be fully appreciated, a better understanding must be developed of the changes that occur in sympathetic neuronal function in normotensive

animals maintained on diets of various physiological levels of Na^+, using more direct tests of SNS activity. Therefore, the purpose of the present study was to examine the effect of acute changes in Na^+ intake within a physiological range on NE turnover in brain and several peripheral sympathetically innervated organs namely heart, intercapular brown adipose tissue and kidney. These organs were chosen because effects on sympathetic activity by other dietary manipulation have been reported within such tissues (Johnston and Balachandran 1987). Both Na^+ concentration and duration of Na^+ treatment were varied independently in an attempt to identify the dose-and time-dependent relationships between Na^+ intake and sympathetic activity.

B. Methods

1. Animals and Diets

Four-week-old female normotensive mice (C57 BL/6J, +/+, Jackson Laboratories, Bar Harbor ME.) were singly housed in hanging wire-mesh cages in a temperature-controlled room (23 ± 1° C). A 12-hour light: dark cycle with lights on commencing at 0900 was maintained throughout experiments. Three diets with a range of Na^+ concentrations were used (Tables II-1 and II-2). Dietary Na^+ composition was confirmed by Alberta Agriculture's Food Laboratory Services Branch. Mice were fed diets varying in Na^+ content for different treatment durations as follows: 0.10% Na^+ or 3.05% Na^+ for 1 day (Exp 1); 0.05% Na^+ or 1.00% Na^+ for 5 days (Exp 2); 0.05% Na^+ or 3.00% Na^+ for 5 days (Exp 3). The diet containing 3.05% Na^+ in Exp 1 was adjusted to 3.00% for all subsequent experiments where this high Na^+ diet was fed.

In each experiment 36 mice were used. Body weight, food intake corrected for spillage, and deionized water intake were measured daily between 0900 and 1200. The mice were adapted for one week to the low Na^+ (0.01% Na^+, Exp 1; 0.05% Na^+, Exp 2,3) purified diet containing approximately 20% of metabolizable energy as protein, 60% as carbohydrate and 20% as fat (Table II-1). The low Na^+ diet of 0.05% Na^+ was employed because it provided the minimum requirement of Na^+ for rats (and presumably mice) (Subcommittee on Laboratory Animal Nutrition, National Research Council, 1972).

2. Experimental Protocol

Experiment 1

After one week of *ad libitum* feeding of the 0.10% Na^+ diet, 18 of the 36 mice were switched randomly to a high Na^+ diet of 3.05% Na^+ for one day (Table II-1); the rest continued feeding on the 0.10% Na^+ diet. Mice fed the high and low Na^+ diets were fed 2.85g and 3.07g of diet/day respectively, to provide equal caloric intake between treatments. The energy intake was calculated from average *ad libitum* intakes obtained during adaptation to the 0.10% Na^+ diet for mice fed the low Na^+ diet and increased for the 3.05% Na^+ diet to provide equal caloric intake. Mice within both treatment groups consumed all food offered.

Mice were killed 24 h after assignment to the experimental groups. Mice were killed by decapitation beginning at 0900h at 0, 3, and 6 hours after i.p. injection with α-methyl-p-tyrosine in physiological saline (α-methyl-D, L-p-tyrosine methyl ester hydrochloride, Sigma Chemical Co. St. Louis MO.; 400 mg/kg body weight). Brain dissected into left and right cerebral hemispheres, heart, kidney with capsule removed, and interscapular brown adipose tissue (IBAT) were rapidly removed, wrapped in aluminum foil, weighed and immediately frozen on dry ice. Tissues were held at -40°C for subsequent determination of NE within one month.

TABLE II-1

Diet constituents

Constituent	g Constituent/100 g Diet				
	Low Sodium (0.05% Na^+) Diet	Low Sodium (0.10% Na^+) Diet	High Sodium (1.00% Na^+) Diet	High Sodium (3.00% Na^+) Diet	High Sodium (3.05% Na^+) Diet
casein	23.0	23.0	23.0	23.0	23.0
mineral mix AIN-76 [1]	0.0	3.5	3.5	3.5	3.5
vitamin mix AIN-76 [2]	1.0	1.0	1.0	1.0	1.0
choline bitartrate	0.2	0.2	0.2	0.2	0.2
cellulose	4.0	4.0	4.0	4.0	4.0
corn oil	10.0	10.0	10.0	10.0	10.0
starch	58.3	58.3	56.0	51.1	50.9
NaCl	0.0	0.0	2.3	7.2	7.4
unique mineral mix [3]	3.5	0.0	0.0	0.0	0.0
protein (%) [4]	19.8	19.8	20.3	21.4	21.4
carbohydrate (%)	57.8	57.8	56.9	54.7	54.5
fat (%)	22.3	22.3	22.8	24.1	24.1
energy density [5]	4.0	4.0	3.9	3.7	3.7

1 AIN-76 mineral mixture (ICN Nutritional Biochemicals, Cleveland, OH)
Composition in g/kg: Calcium phosphate, dibasic 500; sodium chloride 74; potassium citrate, monohydrate 220; potasssium sulfate 52; magnesium oxide 24; manganous arbonate 3.5; ferric citrate 6; zinc carbonate 1.6; cupric carbonate 0.3; potassium iodate 0.01; sodium selenite 0.01; chromium potassium sulfate 0.55; and sucrose 118.03.

2 AIN-76 vitamin mixture (ICN Nutritional Biochemicals, Cleveland, OH)
Composition in mg/kg: thiamin HCl 600; riboflavin 600; pyridoxine HCl 700; nicotinic acid 3000; D-calcium pantothenate 1600; folic acid 200; D-biotin 20; cyanocobalamin 1; retinyl palmite 800; d,l-a-tocopheryl acetate 20000; cholecalciferol 2.5; menaquinone 5, and sucrose 972474 5

3 Refer to Table II-2 for constituents.

4 Casein is 87% protein, % is % of metabolizable energy.

5 The energy densities of the diets expressed as kcal/g based on 4, 4 and 9 kcal/g of metabolizable energy from casein, starch and corn oil, respectively.

TABLE II-2

Unique mineral mixture: constituents

Constituent	Constituent/100g Mineral Mix
calcium phosphate dibasic [1]	50.00
NaCl	3.24
potassium citrate monohydrate	22.00
potassium sulfate	5.2
magnesium oxide	2.4
manganese carbonate	0.35
ferric citrate	0.60
zinc carbonate	0.16
cupric carbonate	0.03
potassium iodate	0.001
sodium selenite	0.001
chromium potassium sulfate	0.055
sucrose	15.96

[1] Source of each ingredient with the exception of sucrose: Fisher Scientific, Edmonton, Alberta

Experiment 2

Mice were adapted for one week to the purified diet containing 0.05% Na^+ by weight (Table II-1). Eighteen mice were then randomly assigned to a 1.00% Na^+ diet for 5 days while the remainder continued to be fed on the 0.05% Na^+ diet, both ad-libitum. On the sixth day of the experiment, NE turnover was determined in brain, heart, kidney and IBAT as in Experiment 1.

Experiment 3

Mice were adapted for one week to the purified diet containing 0.05% Na^+ and then were randomly fed diets containing either 0.05% Na^+ or 3.0% Na^+ for 5 days, both ad-libitum. NE turnover was determined in brain, heart, kidney, and IBAT as in Experiments 1 and 2.

3. Analytical Methods and Statistical Analysis

Analytical Methods

NE was extracted onto alumina under alkaline conditions from the tissue homogenate supernatant by a previously reported method (Bioanalytical Systems, 1980; Appendix 1). Separation of NE was achieved by high performance liquid chromatography (Model 2000, Varian Canada Inc., Georgetown, Ontario) with detection by electrochemical detection (LC 4B, Bioanalytical Systems Inc. Lafayette, IN.), using a mobile phase of 0.075M monochloroacetic acid with 250 mg/l sodium octyl sulphate as an ion-pairing agent, delivered across a reversed-phase analytical column at a flow rate of 1.7 ml/min.

Statistical Analysis

NE turnover rate was calculated by linear regression of the log NE concentration versus the time periods specified (0,3,6 hours) and the slopes were compared using the

variance estimated for difference between slopes (Gill, 1978). Slopes of the regression lines were calculated by the least squares method and were plotted with averaged log tissue NE concentrations, semi-logarithmically. Total NE turnover rate was calculated as the product of the fractional turnover rate and the estimated endogenous NE concentration at time 0 hours for each organ (Brodie et al. 1966). Fractional turnover was expressed as %/h with standard error relative to that of the slope. All values are expressed as means ± SEM. The student's t test was used for group comparisons between treatments where appropriate.

C. Results

Experiment 1

Body weight did not differ between mice fed the 0.10% Na^+ and 3.05% Na^+ diets for one day, subsequent to one week of adaptation to the low Na^+ diet (15.25g ± 0.10 and 16.31g ± 0.10, respectively). Excessive spillage from mice water bottles during cage transport and α-methyl-p-tyrosine injection procedures prevented water intake from being monitored. Endogenous tissue NE concentrations, fractional NE turnover, and total NE turnover did not differ between treatments in any of the organs analyzed (Table II-3, Figure II-1).

Experiment 2

Body weight did not differ between mice fed the 0.05% or 1.00% Na^+ diets for 5 days (16.3g ± 0.1 in 0.05% Na^+ fed vs 16.1g ± 0.1 in 1.00% Na^+ fed mice). Food intake did not differ between mice fed the two diet treatments (Table II-4). Water intake, however, was significantly greater on Days 1 and 2 in mice fed the 1.00% Na^+ diet compared to those fed 0.05% Na^+ (Table II-4). Tissue NE concentration and NE turnover rate in brain, heart, kidney, and IBAT did not differ between mice fed 0.05% Na^+ or 1.00% Na^+ for 5 days (Table II-5, Figure II-2).

Experiment 3

Body weight did not differ between mice fed 0.05% Na^+ or 3.00% Na^+ for 5 days (15.1g ± 0.1 and 13.9g ± 0.1, respectively). Food intake was lower in 3.00% Na^+-fed mice on Days 1 and 3 when compared to 0.05% Na^+ controls but did not differ for 2 days prior to the measurement of NE turnover (Table II-6). Water intake was approximately 40% greater in 3.00% Na^+-fed mice than in 0.05% Na^+-fed counterparts and was consistently different between treatments (Table II-6). IBAT organ weight was found to be significantly lower in 3.00% Na^+-fed mice when compared to low Na^+-fed mice (Table II-7). Tissue NE concentration and NE turnover did not differ between mice fed 0.05% or 3.00% Na^+ for 5 days (Table II-7, Figure II-3).

TABLE II-3

Effects of 0.10 or 3.05% sodium intake for 1 day on tissue norepinephrine turnover: Exp 1

	Organ Weight (mg)	Endogenous NE (nmol/g)	Fractional NE Turnover (%/h)	Total NE Turnover (nmol/g/h)
BRAIN				
0.10% Na^+	200.5 ± 1.1 [1]	2.55 ± 0.05 [2]	17.33 ± 0.75	0.428
3.05% Na^+	184.8 ± 1.7	2.49 ± 0.18	18.01 ± 0.97	0.436
HEART				
0.10% Na^+	77.4 ± 0.5	3.82 ± 0.23	25.78 ± 0.01	1.024
3.05% Na^+	83.0 ± 0.6	4.01 ± 0.20	36.78 ± 0.03	1.727
KIDNEY				
0.10% Na^+	94.1±0.8	4.04 ± 0.41	22.29 ± 1.46	0.931
3.05% Na^+	98.7±0.6	4.99 ± 0.55	16.16 ± 1.16	0.775
IBAT				
0.10% Na^+	100.8 ± 1.0	3.04 ± 0.44	27.64 ± 0.02	0.672
3.05% Na^+	96.7 ± 2.3	3.15 ± 0.43	23.52 ± 0.04	0.493

[1] Values are means ± SEM for n=18 mice in each treatment group.
[2] n=6 mice per time point.

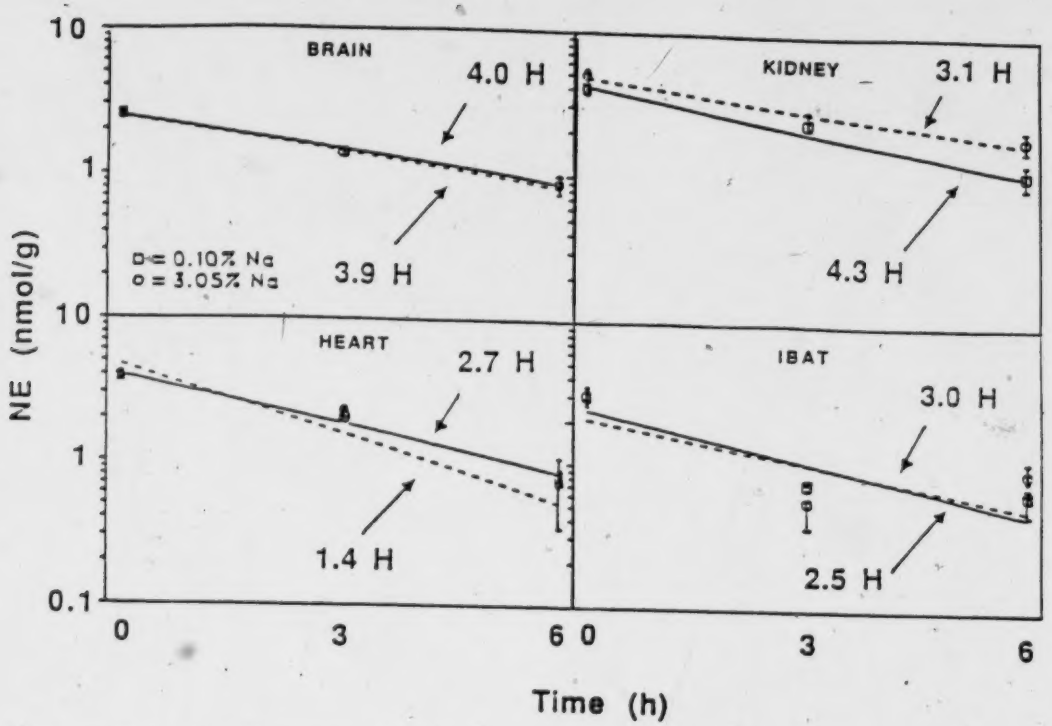

Figure II-1 Disappearance of NE from mouse brain, heart, kidney, and interscapular brown adipose tissue (IBAT) after α - methyl-p- tyrosine administration in mice fed a 20% protein diet containing 0.10% Na^+ (—) or 3.05% Na^+ (- - -) for 1 day (Exp 1). Each point represents mean ± SEM of 5 - 6 mice. Slopes of the two lines within each treatment did not differ in any of the organs analyzed ($p>0.05$). The numbers shown are half-times for disappearance of NE in hours.

Equations for least squares fit of log transformed concentrations [y=log NE(pmol/g); x=time(h)]:

Brain:	0.10% Na^+	y=3.393 - 0.075x	; r= -0.933
	3.05% Na^+	y=3.384 - 0.078x	; r= -0.901
Heart:	0.10% Na^+	y=3.594 - 0.011x	; r= -0.946
	3.05% Na^+	y=3.672 - 0.016x	; r= -0.798
Kidney:	0.10% Na^+	y=3.621 - 0.097x	; r= -0.856
	3.05% Na^+	y=3.681 - 0.070x	; r= -0.842
IBAT:	0.10% Na^+	y=3.386 - 0.120x	; r= -0.814
	3.05% Na^+	y=3.322 - 0.102x	; r= -0.581

TABLE II-4

Food and water intake of mice fed 0.05 or 1.00% sodium diets for 5 days: Exp. 2

Food (g/24 h)	Day 1	2	3	4	5
0.05% Na^+	2.9 ± 0.1 [1]	2.5 ± 0.1	2.6 ± 0.1	2.4 ± 0.2	2.5 ± 0.1
1.00% Na^+	2.7 ± 0.1	2.4 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.3
Water (ml/24 h)					
0.05% Na^+	3.9 ± 0.2	3.7 ± 0.2	4.3 ± 0.3	4.3 ± 0.3	
1.00% Na^+	4.8 ± 0.2*	4.4 ± 0.3*	4.4 ± 0.3	4.3 ± 0.3	

[1] Values are means ± SEM, n=18.

* different from mice fed 0.05% Na^+, $p < 0.05$.

TABLE II-5

Effects of 0.05 or 1.00% sodium intake for 5 days on tissue norepinephrine turnover: Exp 2

	Organ Weight (mg)	Endogenous NE (nmol/g)	Fractional NE Turnover (%/h)	Total NE Turnover (nmol/g/h)
BRAIN				
0.05% Na^+	202.4 ± 0.9 [1]	2.85 ± 0.06 [2]	12.05 ± 0.63	0.331
1.00% Na^+	204.1 ± 0.7	3.01 ± 0.06	12.81 ± 0.56	0.367
HEART				
0.05% Na^+	84.7 ± 0.5	4.32 ± 0.23	19.62 ± 0.98	0.807
1.00% Na^+	84.4 ± 0.4	4.00 ± 0.23	22.01 ± 1.29	0.896
KIDNEY				
0.05% Na^+	98.8 ± 0.8	4.35 ± 0.12	23.07 ± 1.55	0.951
1.00% Na^+	94.6 ± 0.5	4.34 ± 0.25	22.55 ± 1.00	0.909
IBAT				
0.05% Na^+	124.9 ± 1.3	2.75 ± 0.30	17.59 ± 1.59	0.420
1.00% Na^+	123.8 ± 1.9	2.32 ± 0.17	19.46 ± 1.74	0.374

[1] Values given are means ± SEM for n=17-18 mice in each treatment group.
[2] n=5-6 mice per time point.

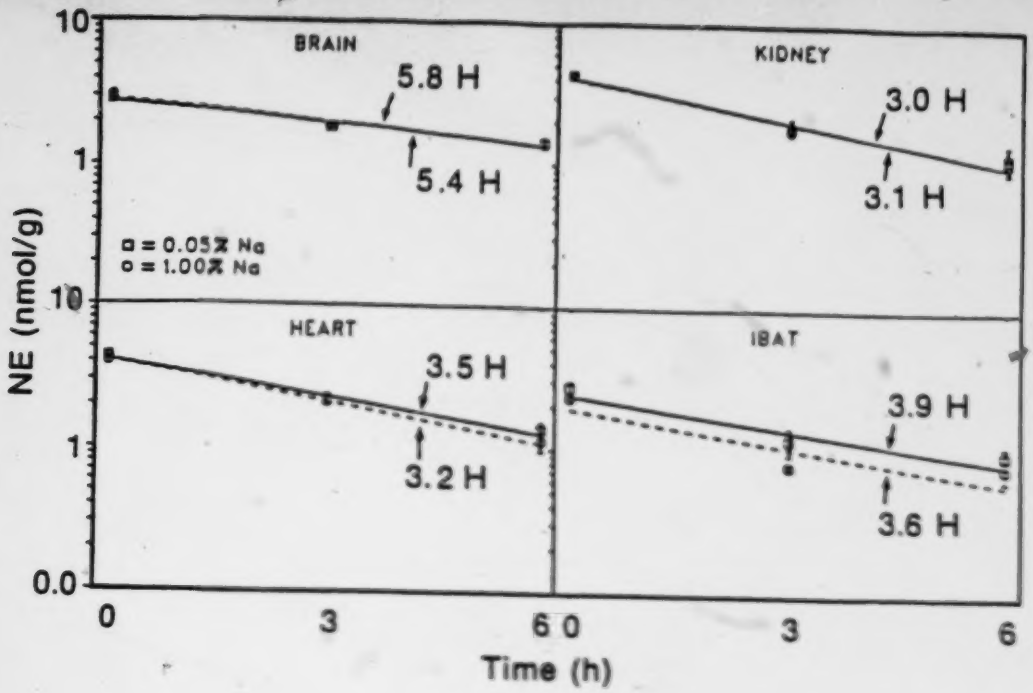

Figure II-2 Disappearance of NE from mouse brain, heart, kidney, and interscapular brown adipose tissue (IBAT) after α - methyl-p- tyrosine administration in mice fed a 20% protein diet containing 0.05% Na^+ (—) or 1.00% Na^+ (- - -) for 5 days (Exp 2). Each point represents mean ± SEM of 5 - 6 mice. Slopes of the two lines within each treatment did not differ in any of the organs analyzed ($p>0.05$). The numbers shown are half-times for disappearance of NE in hours.

Equations for least squares fit of log transformed concentrations [y=log NE(pmol/g); x=time(h)]:

Brain:	0.05% Na^+	y=3.439 - 0.052x	; r= -0.902
	1.00% Na^+	y=3.457 - 0.056x	; r= -0.931
Heart:	0.05% Na^+	y=3.614 - 0.085x	; r= -0.908
	1.00% Na^+	y=3.610 - 0.096x	; r= -0.887
Kidney:	0.05% Na^+	y=3.615 - 0.100x	; r= -0.858
	1.00% Na^+	y=3.605 - 0.098x	; r= -0.930
IBAT:	0.05% Na^+	y=3.378 - 0.076x	; r= -0.768
	1.00% Na^+	y=3.284 - 0.084x	; r= -0.804

TABLE II-6

Food and water intake of mice fed 0.05 or 3.00% sodium diets for 2 days: Exp 3

Food (g/24 h)	Day 1	2	3	4	5
0.05% Na^+	2.7 ± 0.2 [1]	2.6 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	2.6 ± 0.2
3.00% Na^+	2.1 ± 0.3*	2.3 ± 0.2	2.1 ± 0.2*	2.3 ± 0.2	2.8 ± 0.2
Water (ml/24 h)					
0.05%	3.7 ± 0.3	4.1 ± 0.2	4.1 ± 0.2	5.0 ± 0.3	
3.00%	5.2 ± 0.3*	5.9 ± 0.3*	5.8 ± 0.3*	6.9 ± 0.3*	

[1] Values are means ± SEM, n=18

* different from mice fed 0.05% Na^+, $p < 0.05$

TABLE II-7

Effects of 0.05 or 3.00% sodium intake for 5 days on tissue norepinephrine turnover: Exp 3

	Organ Weight (mg)	Endogenous NE (nmol/g)	Fractional NE Turnover (%/h)	Total NE Turnover (nmol/g/h)
BRAIN				
0.05% Na^+	203.5 ± 0.8 [1]	4.63 ± 0.22 [2]	10.03 ± 0.69	0.439
3.00% Na^+	200.0 ± 1.0	5.15 ± 0.24	11.01 ± 1.14	0.523
HEART				
0.05% Na^+	79.8 ± 0.3	7.46 ± 0.27	20.14 ± 0.79	1.461
3.00% Na^+	73.2 ± 0.2	6.90 ± 0.34	20.70 ± 1.60	1.434
KIDNEY				
0.05% Na^+	90.3 ± 0.4	6.12 ± 0.27	21.55 ± 1.50	1.152
3.00% Na^+	93.2 ± 0.5	6.02 ± 0.33	23.09 ± 1.93	1.332
IBAT				
0.05% Na^+	138.5 ± 1.4	3.89 ± 0.21	19.72 ± 1.68	0.627
3.00% Na^+	83.5 ± 1.0 *	5.94 ± 0.75	23.52 ± 2.35	1.130

[1] Values are means ± SEM for n=18 mice in each treatment group.
[2] n=6 mice per time point.
* different from mice fed 0.05% Na^+, $p<0.001$

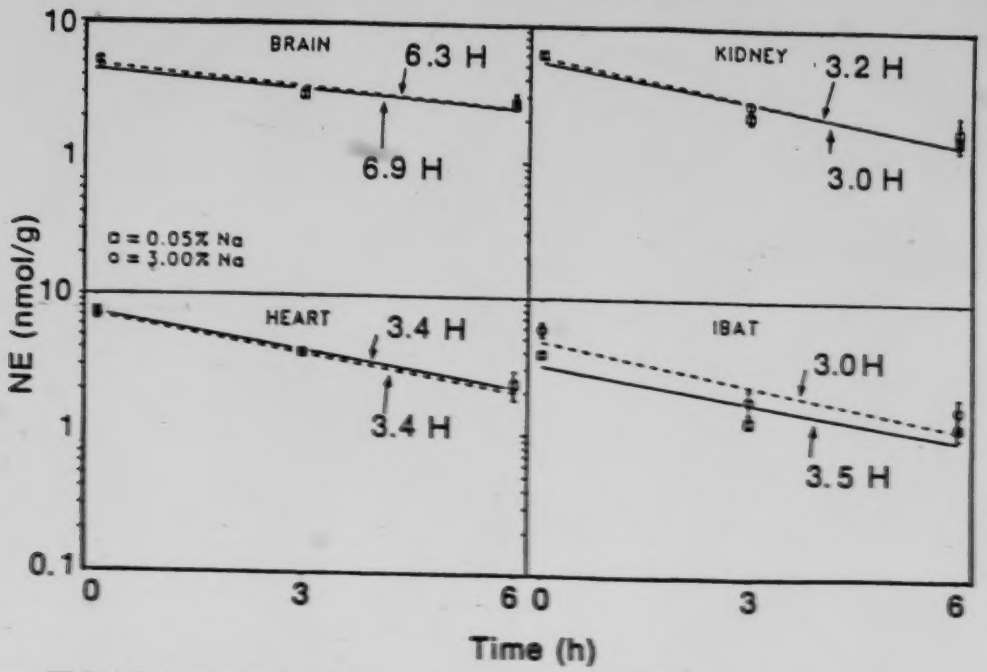

Figure II-3 Disappearance of NE from mouse brain, heart, kidney, and interscapular brown adipose tissue (IBAT) after α - methyl-p- tyrosine administration in mice fed a 20% protein diet containing 0.05% Na^+ (—) or 3.00% Na^+ (- - -) for 5 days (Exp 3). Each point represents mean ± SEM of 5 - 6 mice. Slopes of the two lines within each treatment did not differ in any of the organs analyzed ($p>0.05$). The numbers shown are half-times for disappearance of NE in hours.

Equations for least squares fit of log transformed concentrations: [y=log NE(pmol/g); x=time(h)]:

Brain:	0.05% Na^+	y=3.641 - 0.044x	; r= -0.843
	3.00% Na^+	y=3.676 - 0.048x	; r= -0.772
Heart:	0.05% Na^+	y=3.861 - 0.087x	; r= -0.908
	3.00% Na^+	y=3.841 - 0.090x	; r= -0.887
Kidney:	0.05% Na^+	y=3.728 - 0.094x	; r= -0.842
	3.00% Na^+	y=3.761 - 0.100x	; r= -0.802
IBAT:	0.05% Na^+	y=3.502 - 0.086x	; r= -0.768
	3.00% Na^+	y=3.681 - 0.102x	; r= -0.803

D. Discussion

The results of this study indicate that acute increases in dietary Na^+ within a physiological range do not affect NE turnover in normotensive mice. Increases in Na^+ intake from the requirement level of 0.05% to 1.00% (Exp 2) or 3.00% (Exp 3) for 5 days did not affect NE turnover in the brain or peripheral sympathetically innervated tissues. Similarly, an acute increase in Na^+ intake from 0.10% to 3.05% for one day did not affect NE turnover in brain, heart, kidney, or IBAT (Exp 1).

These findings are relevant for several reasons. This is the first report of the effects of acute increases in Na^+ intake on NE turnover. Secondly, these findings examined separately the effects of dietary Na^+ concentration at 4 levels of intake and over two treatment durations. Further, Na^+ concentrations employed were within a physiological range, eliminating a potentially toxic pharmacological effect that extreme Na^+ intakes may have on sympathetic response.

Results are contrary to those reported with chronic Na^+ supplementation in normotensive rats wherein a negative relationship between Na^+ intake and sympathetic activity was observed (Kaufman and Vollmer 1984; Dietz et al. 1980; Saavedra et al. 1983). Specifically, rats in these studies were generally at extremes of Na^+ intake consuming 6.87% or 8.0% Na^+-supplemented purified diets (Kaufman and Vollmer 1984) or were controls consuming a deficient 0.02% Na^+ diet (Kaufman and Vollmer 1984; Dietz et al. 1980; AIN Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies, 1977). Thus the inverse effect of Na^+ intake on sympathetic activity apparent through their measurement of plasma or tissue NE concentrations was not supported by our study. Our results are consistent with recent findings of Dawson and Oparil (1987) who reported no effect on renal NE concentration when dietary Na^+ increased from a 'normal' level, assumed physiological, to 3.4% Na^+ for two weeks.

Variation in treatment duration from 1 to 5 days, regardless of dietary Na^+ concentration within a physiological range, did not affect central or peripheral NE turnover (Exp 1, Exp 3). It remains possible that noradrenergic responses to alterations in dietary Na^+ are temporal in nature and are dependent on time as well as dose of Na^+ treatment. Specifically, treatment duration in excess of 5 days or even between 2 and 5 days, may be required to elicit a noradrenergic response in normotensive mice to increased Na^+ intake within a physiological range. Alternatively, sympathetic activity may respond to more extreme changes in Na^+ intake only, and/or to treatment duration.

NE concentration declined monoexponentially in all tissues examined except IBAT demonstrating that one of the major assumptions of steady-state kinetics had been met. The decline in NE concentration in IBAT apperared to be biphasic and may underestimate NE turnover rate. These results are similar to those reported in a previous study where the fit of the data for the monoexponential curve was less significant in IBAT than in other organs studied when NE concentration was determined at seven time points over a 6 hour period (Johnston and Balachandran 1987).

Experimental food and water intake data suggest Na^+ and fluid balance is altered in response to increased dietary Na^+ from 0.05% or 1.00% (Exp 2) or 3.00% (Exp 3). Observed increases in water intake in response to altered dietary Na^+ suggest enhancement of natriuretic and diuretic processes. Whether these processes and their concomitant effect on Na^+ balance are associated with altered noradrenergic activity chronically remains to be determined. Recent studies demonstrate that Na^+ and fluid homeostasis regulation is linked to noradrenergic activity through α-adrenergic control of renal filtration rate and β-adrenergic control of renin release (Hall et al. 1986; DiBona 1986). Efferent renal sympathetic activity has been shown to undergo appropriate changes to facilitate renal Na^+ conservation during Na^+ deficit and to promote Na^+ excretion during Na^+ excess through increased or decreased renal efferent arteriolar constriction, respectively (DiBona 1986). An inverse relation between Na^+ and free NE excretion in chronically denervated one

kidney rats has been reported by Morgunov and Baines (1981) and between Na^+ intake and urinary NE in normotensive human studies by Alexander et al. (1974) who reported free NE excretion decreased 44% when Na^+ intake increased from 9 to 209 or 259 mmol/day for 10 days. Therefore, it is possible that more prolonged consumption of a high Na^+ diet is necessary to elicit a sympathetic response to altered Na^+ intake, for maintenance of Na^+ balance.

More studies investigating the individual effects of dietary Na^+ concentration, Na^+ balance, and Na^+ treatment duration on SNS activity in normotensive animals are needed to clarify the association between dietary Na^+ and sympathetic activity and so characterize the possible role of enhanced noradrenergic activity in the development of hypertension.

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III. Effects of Subchronic and Chronic Increases in Sodium Intake on Sympathetic Nervous System Activity: A Temporal Study

A. Introduction

There is increasing evidence that high sodium (Na^+) intakes are associated with increased sympathetic nervous system (SNS) activity, as measured by plasma concentration and urinary excretion of catecholamines in normotensive humans (Luft et al. 1979; Parfrey et al. 1981; Romoff et al. 1979; Dustan 1985; Campese et al. 1982; Berglund 1983; Nicholls et al. 1980). For example Nicholls et al. (1980) showed that when Na^+ intake increased from 120 to 240 mmol/day, plasma norepinephrine (NE) increased approximately 50%. Thus hypertension associated with high Na^+ intakes may be attributed to a maladaptive response wherein dietary Na^+ enhances SNS activity leading to reduced Na^+ excretion and increased vascular resistance through alpha-adrenergic actions (Scribner 1974; Hall et al. 1986; Vollmer 1984).

Experiments investigating the effects of increasing dietary Na^+ on noradrenergic activity in hypertensive animals provide consistent evidence that a positive correlation between Na^+ intake and SNS activity exists (Battarbee et al. 1979; Winternitz and Oparil 1982; Dietz et al. 1980). In spontaneously hypertensive rats, an increased Na^+ intake has been shown to increase plasma NE concentration, increase urinary NE excretion and accelerate the onset and severity of blood pressure elevation (Winternitz and Oparil 1982; Dietz et al. 1980). Similarly, Dietz et al. (1980) found sympathetic activity as assessed by plasma NE concentration, to increase with Na^+ loading in spontaneously hypertensive rats.

Limited studies have been conducted to determine the site(s) at which sympathetic function is altered by dietary Na^+ intake. The effect that a high Na^+ diet has on tissue noradrenergic activity is not clear. In several hypertensive models, a high Na^+ intake has been reported to increase sympathetic neuronal activity assessed through determination of

NE turnover rate in cerebral hemispheres (Gradin et al. 1985), the pons (de Champlain and Van Ameringen 1972), or the heart, intestine, and spleen of DOCA-Na^+ hypertensive rats (de Champlain et al. 1969).

Normotensive animal studies investigating the relationship between dietary Na^+ and SNS function are limited and provide inconsistent evidence that a negative correlation between Na^+ intake and sympathetic activity exists. A five week increase in Na^+ intake from 0.02% to 0.66% Na^+ was reported to decrease kidney, heart, and arterial tissue NE concentrations in rats (Kaufman and Vollmer 1984). Similarly, de Champlain et al. (1969) reported increased NE turnover in heart, intestine, and spleen of normotensive rats when fed a 1% Na^+ chloride diet for four to six weeks. In contrast, Na^+ loading has been shown to increase adrenal NE concentration in Dahl Na^+-resistant rats (Saavedra et al. 1983). Increased Na^+ intake from 0.02% to 6.87% Na^+ was shown not to affect endogenous tissue NE concentrations by Kaufman and Vollmer (1984). Three week Na^+ loading has been shown to exert opposite effects on NE turnover in rabbit cardiovascular tissues and brain stem (Tanaka et al. 1982). Conflicting results between studies may be attributed to intra- and interspecies variation in noradrenergic response to alteration in Na^+ intake or to differences in methods of SNS assessment. Alternatively, discrepancies may be attributed to the application of extreme, variable dietary Na^+ concentration, and the confounding effects of variation in treatment duration on sympathetic response. A more complete assessment of the effect of Na^+ intake on SNS activity would involve studying the independent effects of dietary Na^+ concentration and Na^+ treatment duration on noradrenergic activity.

The purposes of the present study were first to determine the time-dependent relationship between Na^+ intake, NE and DA excretion, and NE turnover in heart and kidney and second to determine the effects of physiological levels of Na^+ intake chronically on NE turnover in brain and several peripheral sympathetically innervated organs, namely heart, IBAT and kidney. Organ specific NE turnover and urinary excretion of NE were

used to assess noradrenergic activity in response to altered Na^+ intake to characterize the relationship between Na^+ treatment duration and sympathetic activity.

B. Methods

1. Animals and Diets

Three experiments were conducted using four-week-old female normotensive mice (C57 BL/6J. +/+, Jackson Laboratories, Bar Harbor ME). In all experiments Na^+ intake was increased from 0.05% to 3.00% Na^+, composition as previously reported (Table II-1, Table II-2). Treatment duration was varied to characterize the time-dependent relationship between increased Na^+ intake and sympathetic activity. Mice were fed *ad libitum* as follows: 0.05% Na^+ or 3.00% Na^+ for 10 days (Exp 1); 0.05% Na^+ or 3.00% Na^+ for 13 days (Exp 2); 0.05% Na^+ or 3.00% Na^+ for 30 days (Exp 3). Low (0.05%) and high (3.00%) Na^+ diets contained approximately 20% of metabolizable energy as protein, 60% as carbohydrate and 20% as fat (Table II-1). The low Na^+ diet of 0.05% Na^+ was employed because it provided the minimum requirement of Na^+ for rats and presumably mice. (Subcommittee on Laboratory Animal Nutrition, National Research Council, 1972). Dietary Na^+ content was confirmed by Alberta Agriculture's Food Laboratory Services Branch. Mice had *ad libitum* access to deionized water. A 12-hour light: dark cycle with lights on commencing at 0900 and a room temperature of $23 \pm 1°$ C was maintained throughout experiments.

2. Experimental Protocol

Experiment 1 Protocol

Urinary excretion of Na^+, free NE and DA and creatinine were measured in mice fed a low (0.05%) or high (3.00%) Na^+ diet to determine their temporal adaptation to Na^+ intake. Forty-eight mice were adapted to the 0.05% Na^+ diet gelatinized by the addition of 0.03% agar (Agar Purified, Difco Laboratories, Detroit, MI) with a diet-to-water ratio of 1.2:1 for one week in metabolism cages containing 4 mice per cage after which they either continued to receive the 0.05% Na^+ diet or were fed a 3.00% Na^+ diet for 10 days. Mice were fed 26.0 g gelatinized high and low Na^+ diets daily (14.28g diet/24h), providing 4.60 mEq and 0.08 mEqNa/24h/4 mice, respectively. Assuming equal food intake among individual mice within each cage, 0.05% and 3.00% Na^+ treatment groups were provided with 0.02 and 1.15 mEqNa/24h/mouse, respectively. Water intake was measured daily. Mice were fed at 2000h to minimize urine contamination by diet. The mice excreted their greatest proportion of urine in the light period, prior to 2000h. Urine was collected prior to 2000h as well as the following morning in n=24 cases, to provide completely uncontaminated samples for estimation of the contribution of dietary Na^+ spillage to urinary NE excretion. Otherwise all urine was collected daily at 0900 h in water cooled, acid-treated (150µl 6 N HCl) collection cups for subsequent determination of 24 h Na^+, free NE, free DA, and creatinine excretion. Urine volume was measured and aliquots were transfered into untreated vials for determination of Na^+ and creatinine, and vials treated with 20µl EDTA/glutathione stabilizing solution and frozen at -40° C until assayed for free NE and DA (Bioanalytical Systems Inc., 1982).

Experiment 2 Protocol

Thirty six mice were housed singly in hanging wire mesh cages and were randomly fed diets containing either 0.05% Na^+ or 3.00% Na^+ for 13 days. Mice were killed by decapitation beginning at 0900h at 0,3, or 6 hours after i.p. injection with α -methyl-p-tyrosine (400 mg/kg body weight) in physiological saline. Kidney with capsule removed and heart were rapidly removed, wrapped in aluminum foil, weighed and immediately frozen on dry ice. Tissues were held at -40° C for subsequent determination of NE within one month.

Experiment 3 Protocol

Forty-eight mice, housed singly in hanging wire mesh cages except for 10 days at the beginning of the experiment when they were housed 4 / metabolism cage (Exp 1), were adapted for one week to a diet containing 0.05% Na^+ (Table III-1). After one week of *ad libitum* feeding of the 0.05% Na^+ diet, 18 of the 36 mice were randomly assigned to the 3.00% Na^+ diet for 30 days, while remaining mice continued feeding on the 0.05% Na^+ diet. Mice were killed by decapitation beginning at 0900h at 0, 3, or 6 hours after i.p. injection with α-methyl-p-tyrosine (400 mg/kg body weight) in physiological saline. Brain dissected into left and right cerebral hemispheres, heart, kidney with capsule removed, and interscapular brown adipose tissue (IBAT) were rapidly removed, wrapped in aluminum foil, weighed and immediately frozen on dry ice. Tissues were held at -40° C for subsequent determination of NE within one month.

3. Analytical Methods and Statistical Analysis

Analytical Methods

Urinary Na^+ was measured by flame emission spectrophotometry (Model SP9, Philips Instruments Ltd., Vancouver, B.C.; Appendix 2). Correction factor for Na^+ spillage was calculated from averaged differences in urinary Na^+ concentration between urine collected at 2000h (prior to feeding) and 0900h (subsequent to feeding) for mice fed the high sodium diet only. Mice fed the low sodium diets consistently showed no food spillage in the urine collection cups.

Creatinine excretion was measured initially to determine the completeness of urine collection. Urinary creatinine was determined colourimetrically by a kit (#555 Sigma Chemical Co., St. Louis, MO., Appendix 3).

Free NE and DA in pooled 24-h-urinary excretions were simultaneously determined by high performance liquid chromatography with electrochemical detection under conditions discussed previously (Chapter II). In brief, 1 or 2 ml samples for quantification of free forms were decanted onto isolation columns (Econo-Column, Bio Rad Laboratories, Richmond, CA) containing cation-exchange resin (Bio Rex 70, Bio Rad Laboratories, Richmond CA., Appendix 4). Eluted NE and DA were subsequently extracted onto alumina under alkaline conditions and were separated chromatographically by a previously reported method (Bioanalytical Systems Inc., 1982).

NE in the tissue homogenate supertnatant adsorbed on alumina and extracted in 0.2M perchloric acid, was assayed by high performance liquid chromatography (Model 2000, Varian Canada Inc. Georgetown, Ontario) with electrochemical detection (Model LC4; Bioanalytical Systems Inc., West Lafayette, IN.) for determination of NE turnover rate in Experiments 2 and 3 (Appendix 1).

Statistical Analysis

As creatinine was used initially as an indicator of completeness of urine collection, an analysis of covariance of creatinine with urine volume, Na^+ excretion, free NE and free DA excretion was done. The effects of treatment (0.05% Na^+, 3.00% Na^+ diets) and day (1 through 10) and their interactions on urine volume Na^+ excretion, creatinine excretion, free NE and free DA excretion were tested by least squares analysis of variance with day treated as a repeated variable (BMDP-2V Program, BMDP Statistical Software Inc., Los Angeles, CA.). Where significant interactions were found, this was followed by a Student Newman Keul's multiple comparison procedure within each treatment to identify where the significant differences between days lay (SPSS Guide to Data Analysis, 1986). A p value <0.05 was considered significant. All urinary parameters are expressed as means ±SEM.

Total NE turnover rate (K) was calculated as the product of the estimated NE concentration at time 0 for each organ and the rate constant (k) where k=slope/0.434 (Brodie et al. 1966). Fractional turnover (kx100%) was expressed as % /h: standard error (SE) of k (SEk) equalled SE for b (SEb) divided by 0.434. Rate of NE turnover was calculated by linear regression of the log NE concentration versus the time periods specified (0, 3, 6 hours) and the slopes were compared using the variance estimated for difference between slopes (Gill 1978). The student's t test was used for group comparisons between treatments where appropriate. Turnover values are expressed as means ± SEM.

C. Results

Experiment 1

Daily water intake was consistently three-fold greater in mice fed the 3.00% Na^+ diet compared to those fed the 0.05% Na^+ diet (23.24 ± 1.63 vs 6.81 ± 0.45 ml/24h; Table III-1). 24h urine volume was also significantly greater in mice fed 3.00% Na^+ diet and was approximately six-fold greater than that seen with mice fed the 0.05% Na^+ diet

when averaged over the 10 day treatment duration (17.03 ±0.91 vs 2.83 ± 0.15 ml/24h; Table III-1).

The contribution of diet spillage to urine Na^+ excretion was 2.24± 0.58 mEq/24h in the mice fed the 3.00% Na^+ diet (n=24). After subtracting this amount from the total Na^+ excretion on the high Na^+ diets, 24 h Na^+ excretion was 44 fold higher than control (1.86 ± 0.31 versus 0.04 ± 0.00 mEqNa/24h) after 1 day on the 3.00% Na^+ diet, peaked at a 140-fold elevation above control on day 3 (7.37 ± 0.047 mEqNa/24h) , and remained elevated throughout the balance of the study (Figure III-1). Average daily Na^+ intake and daily Na^+ excretion were comparable within 0.05% and 3.00% Na^+ treatment groups and were 0.08±0.01 vs 0.05±0.01 mEqNa/24h/4 mice and 4.60±0.32 vs 4.59±0.57 mEqNa/24h/4 mice for the intake and excretion of mice on the respective dietary treatments.

Urinary creatinine excretion/24h was greater in mice fed 3.00% Na^+ than in those fed 0.05% Na^+ on days 4,6,7 and 9 (Figure III-2). Average creatinine excretion was 49% higher in mice fed the 3.00% Na^+ diet than in those fed the 0.05% Na^+ diet (1.68 ± 0.20 vs 1.13 ± 0.14 mg/24h, $p<0.05$). Daily creatinine excretion was constant within treatments and did not differ between days within high or low Na^+ groups (Table III-2,3).

Urinary free NE excretion/24h was significantly lower in mice fed the 0.05% Na^+ diet than in those fed the 3.00% Na^+ diet on days 2,4, and 6 through 10 (Figure III-3). Free NE excretion was 74% lower in mice fed 0.05% Na^+ diets (0.302 ± 0.048 µg/24h) compared to those fed 3.00% Na^+ (0.527 ± 0.038 µg/24h) when averaged over the 10 days. There appeared to be a curvilinear decline in NE excretion with continued consumption of the 0.05% Na^+ diet such that differences between treatments became consistent by day 6, 13 days after initiation of the low-Na^+ feeding regime (Fig III-1). This appeared as a significant day-by-treatment interaction (Table III-2) such that in the mice fed 0.05% Na^+ diets, free NE excretion was significantly lower on days 2 through 10 than on day 1 (by Newman Keul's multiple comparison procedure Table III-3).

Urinary free DA excretion/24h showed a temporal response similar to that of free NE (Figure III-4). Free DA excretion was approximately 52% lower (2.41 ± 0.43 vs 3.59 ± 0.46 µg/24h) in mice fed 0.05% Na^+ diet when averaged over 10 days. Free DA levels were significantly lower in mice fed the 0.05% Na^+ diet on days 4,6,7, and 9 when compared to 3.00% Na^+ counterparts (Figure III-5). The curvilinear decline in free DA excretion in mice fed the 0.05% Na^+ diets appeared statistically as a day by treatment interaction (Table III-2) such that free DA excretion was significantly lower on days 2 and 4 through 10 than on days 1 and 3 (Table III-3).

Analysis of covariance showed creatinine excretion was a covariate for urine volume ($F_{(1,89)}= 24.94$, $p<0.005$), urinary Na^+ ($F_{(1,89)}= 12.45$, $p<0.005$), free NE excretion ($F_{(1,89)}= 15.05$, $p<0.005$) and free DA excretion ($F_{(1,89)}= 5.36$, $p<0.005$). However, when urinary variables were subsequently expressed relative to creatinine the treatment effects remained the same except for DA. Specifically, differences between treatments in Na^+ excretion expressed relative to creatinine were consistent throughout the study and averaged 86 fold lower in 0.05% Na^+-fed mice compared to 3.00% Na^+-fed counterparts (0.05 ± 0.01 vs 4.32 ± 0.26 mEq Na^+/mg creatinine / 24h; Figure III-5). Urinary free NE levels relative to creatinine excretion were significantly lower in 0.05% Na^+-fed mice on days 3, 6, 7, and 10 when compared to 3.00% Na^+-fed mice (Figure III-6). Free DA excretion did not differ between treatments when expressed relative to creatinine but showed a temporal response similar to that when expressed independent of creatinine (Figure III-4 and Figure III-7).

TABLE III-1

Effect of 0.05% and 3.00% sodium intakes on water intake and urine volume: Exp 1

Day	Treatment	Intake ml/24h	Excretion ml/24h
1	0.05% Na^+	4.21 ± 0.37[1]	2.35 ± 0.18[1]
	3.00% Na^+	14.56 ± 1.16*	11.10 ± 1.27*
2	0.05% Na^+	4.47 ± 0.26	2.57 ± 0.32
	3.00% Na^+	23.40 ± 1.34*	15.30 ± 0.87*
3	0.05% Na^+	4.65 ± 0.44	3.78 ± 0.34
	3.00% Na^+	24.65 ± 1.73*	17.07 ± 1.84*
4	0.05% Na^+	6.05 ± 0.24	2.63 ± 0.30
	3.00% Na^+	27.32 ± 1.72*	16.62 ± 1.30*
5	0.05% Na^+	5.78 ± 0.42	3.60 ± 0.54
	3.00% Na^+	21.44 ± 1.85*	16.02 ± 1.75*
6	0.05% Na^+	5.96 ± 0.41	2.35 ± 0.34
	3.00% Na^+	33.89 ± 2.66*	21.32 ±2.51*
7	0.05% Na^+	5.21 ± 0.39	2.58 ± 0.32
	3.00% Na^+	22.96 ± 1.33*	15.43 ± 1.16*
8	0.05% Na^+	5.32 ± 0.41	2.83 ± 0.44
	3.00% Na^+	24.80 ± 1.75*	18.72 ± 1.97*
9	0.05% Na^+	6.07 ± 0.51	2.72 ± 0.50
	3.00% Na^+	20.02 ± 1.11*	20.02 ± 1.11*
10	0.05% Na^+	5.72 ± 0.53	2.88 ± 0.53
	3.00% Na^+	19.35 ± 1.47*	18.68 ± 1.84*

1 Values are means ± SEM; n=6 cages per treatment, 4 mice per cage.

* different from 0.05% Na^+ treatment group ($p<0.01$, Students t-test).

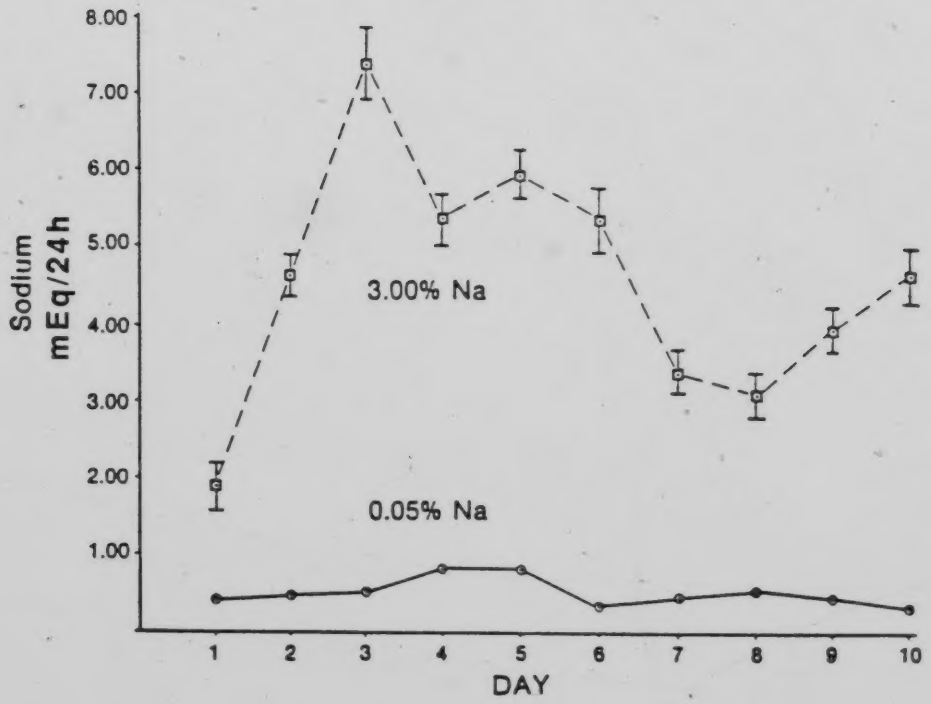

Figure III-1 Average pooled daily Na^+ excretion ±SEM in mice fed 0.05% Na^+ or 3.00% Na^+ diets for 10 days (Exp 1). Values represent mean 24h Na^+ excretions from 6 metabolism cages containing 4 mice per cage. Urinary Na^+ was lower in mice fed 0.05% Na^+ diets than those switched to 3.00% Na^+ throughout the study, all $p < 0.001$.

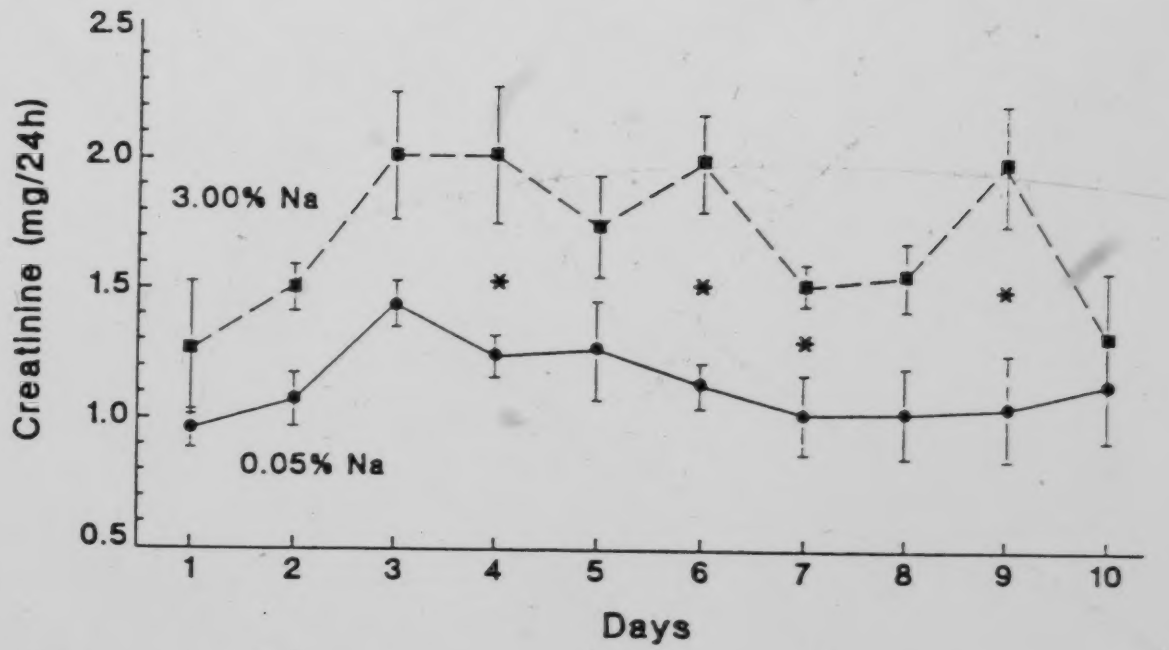

Figure III-2 Total daily creatinine excretion for mice fed 0.05% Na^+ or 3.00% Na^+ diets for 10 days (Exp1). Values shown are means ±SEM for total daily creatinine excretion from 6 metabolism cages per treatment containing 4 mice per cage. Urinary creatinine was higher in mice fed 3.00% Na^+ diet than those fed 0.05% Na^+ diet on Days 4,6,7 and 9 (*$p<0.05$).

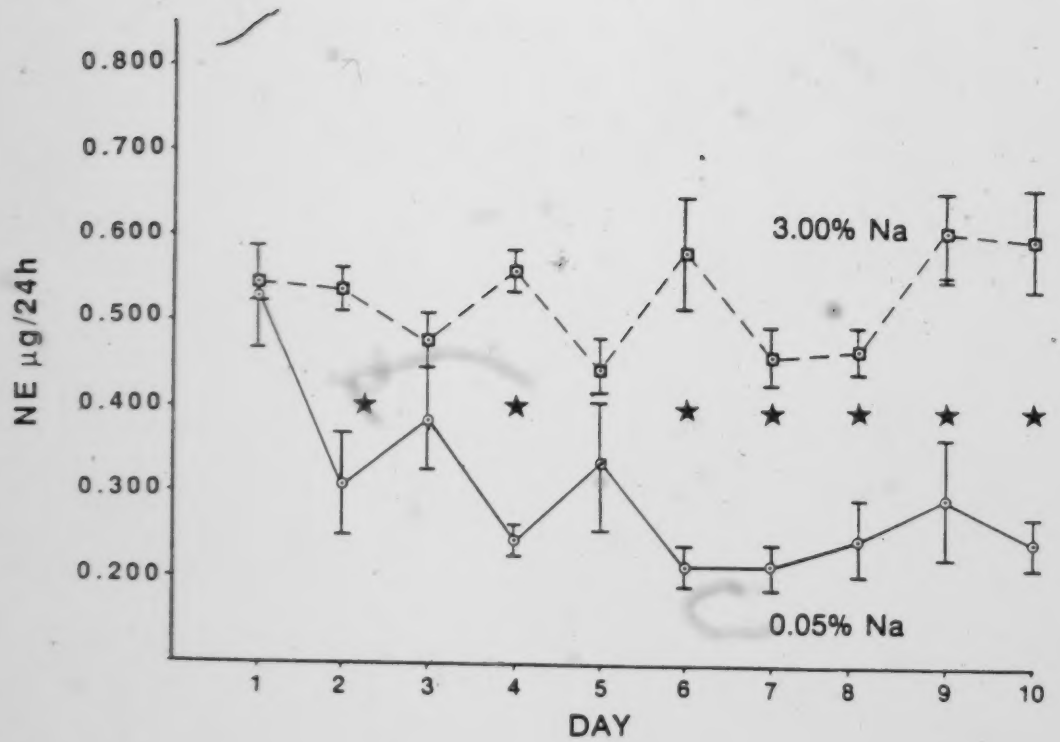

Figure III-3 Daily free NE excretion of mice fed a 0.05% or 3.00% Na^+ diet for 10 days. Values shown are means ±SEM and represent daily free NE from 6 metabolism cages per treatment containing 4 mice per cage. Free NE excretion was lower in mice fed 0.05% Na^+ compared to those fed 3.00% Na^+ on days 6 through 10 , 13 days after initiation of low Na^+ feeding (*$p<0.05$).

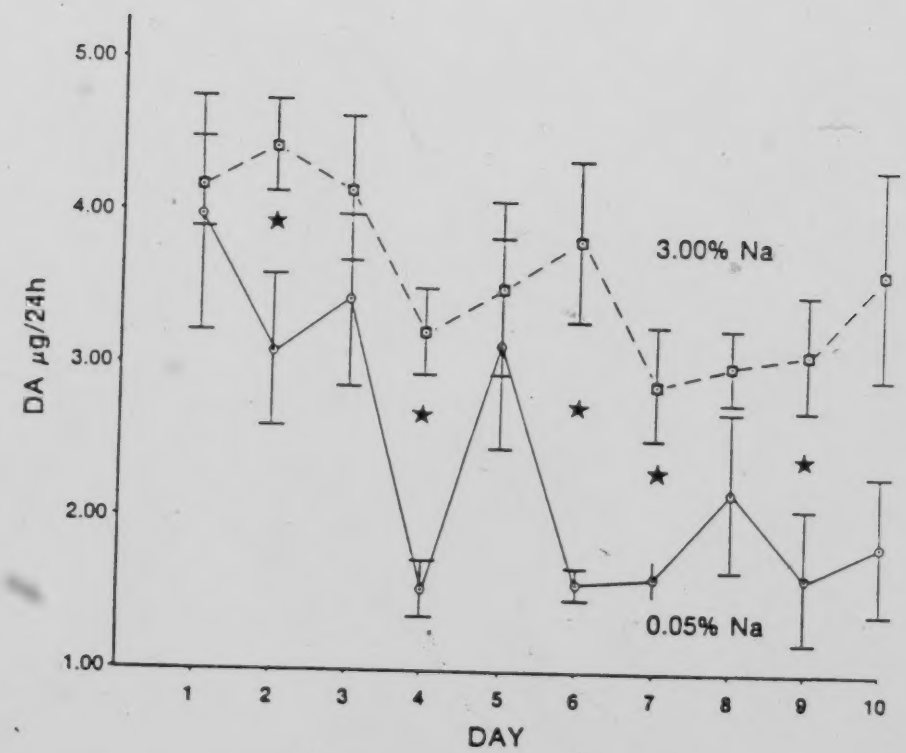

Figure III-4 Daily urinary free DA excretion of mice fed a 0.05% or 3.00% Na^+ diet for 10 days. Values shown are means ±SEM and represent daily free DA excretion from 6 metabolism cages per treatment containing 4 mice per cage. Free DA excretion was significantly lower in low (0.05%) Na^+-fed mice compared to high (3.00%) Na^+-fed counterparts on days 2, 4, 6, 7 and 9 ($p < 0.01$).

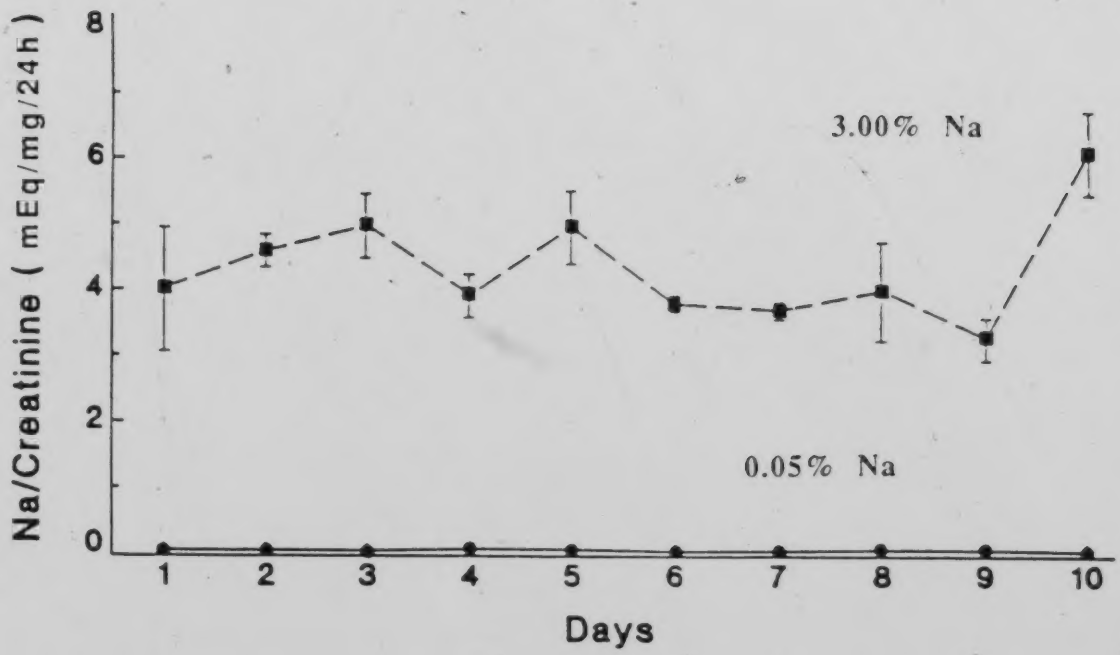

Figure III-5 Na^+ excretion relative to creatinine in mice fed 0.05 or 3.00% Na^+ diets for 10 days. Values shown are means ±SEM and for daily Na^+/creatinine excretion from 5 - 6 metabolism cages per treatment, 4 mice per cage. Average daily Na^+/creatinine excretion from low Na^+-fed mice was 0.0521± 0.005 ($p<0.005$ on all days).

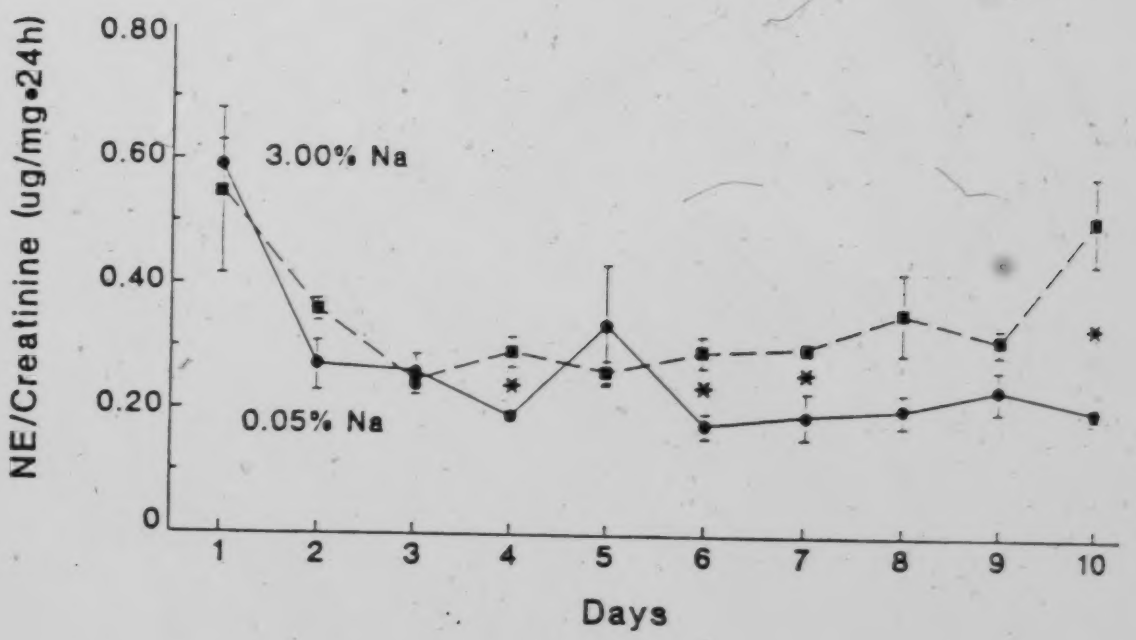

Figure III-6 Daily urinary free NE relative to creatinine excretion for mice fed diets containing 0.05% or 3.00% Na^+. Values shown are means ±SEM for daily free NE/creatinine excretion from 4 - 6 metabolism cages per treatment, 4 mice per cage (*$p<0.01$).

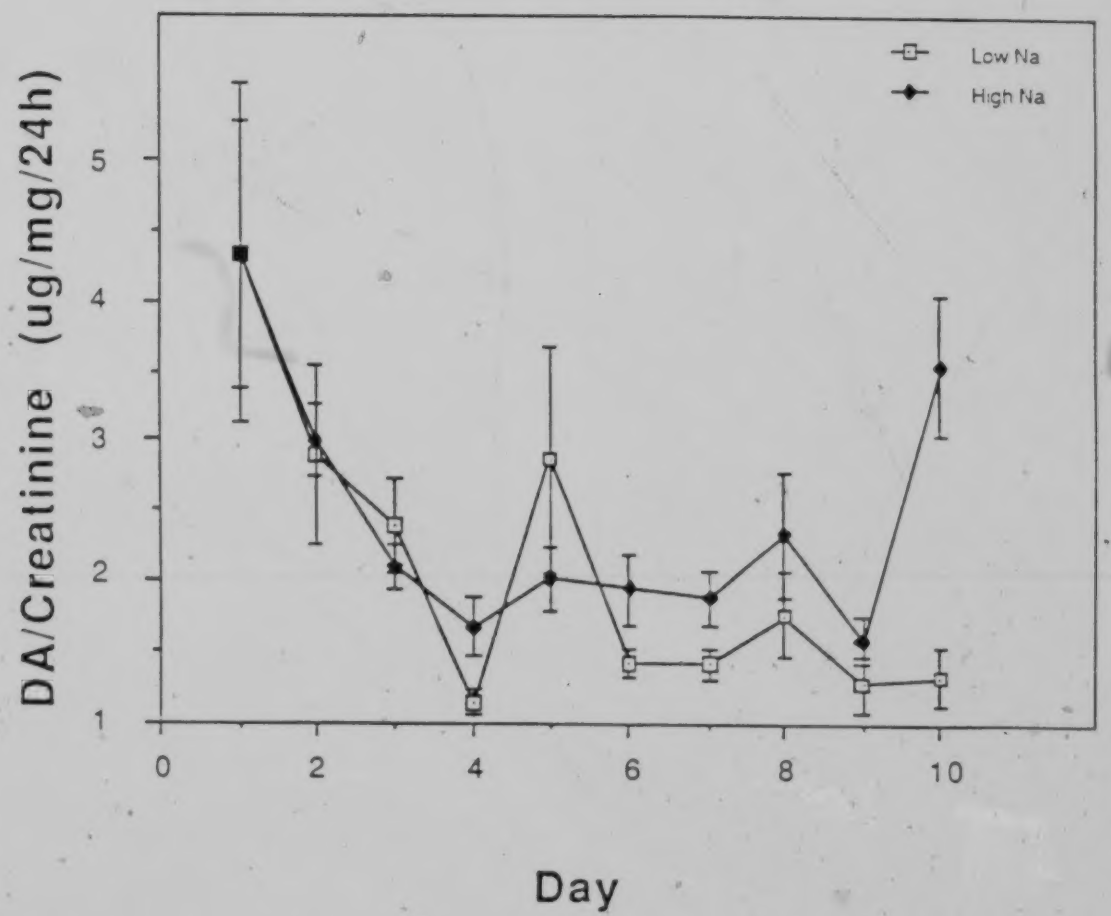

Figure III-7 Daily urinary free DA relative to creatinine excretion for mice fed diets containing 0.05% or 3.00% Na^+. Values shown are means ±SEM for daily free DA/creatinine excretion from 4 - 6 metabolism cages per treatment containing 4 mice per cage (all $p>0.05$).

TABLE III-2

Analysis of variance with day treated as a repeated variable: Exp 1

Urine Volume

	F	df	p
Day	4.50	9	p<0.005
Treatment	186.93	1	p<0.005
Day x Treatment Interaction	4.34	9	p<0.005

Na^+ Excretion

	F	df	p
Day	7.88	9	p<0.005
Treatment	397.32	1	p<0.005
Day x Treatment Interaction	7.77	9	p<0.005

Creatinine Excretion

	F	df	p
Day	2.95	9	p<0.005
Treatment	19.08	1	p<0.005
Day x Treatment Interaction	1.14	9	N.S.

Free NE Excretion

	F	df	p
Day	3.82	9	p<0.005
Treatment	57.56	1	p<0.005
Day x Treatment Interaction	5.21	9	p<0.005

Free DA Excretion

	F	df	p
Day	4.57	9	p<0.005
Treatment	13.55	1	p<0.005
Day x Treatment Interaction	2.03	9	p<0.05

TABLE III-3

Newman-Keul analysis of urinary parameters for significant differences due to day within treatments : Exp 1 [1]

Urine Volume

Day	1	2	3	4	5	6	7	8	9	10
0.05% Na^+										
3.00% Na^+										

Na^+ Excretion

Day	1	2	3	4	5	6	7	8	9	10
0.05% Na^+										
3.00% Na^+										

Creatinine Excretion

Day	1	2	3	4	5	6	7	8	9	10
0.05% Na^+										
3.00% Na^+										

Free NE Excretion

Day	1	2	3	4	5	6	7	8	9	10
0.05% Na^+										
3.00% Na^+										

Free DA Excretion

Day	1	2	3	4	5	6	7	8	9	10
0.05% Na^+										
3.00% Na^+										

[1] Any two means not underscored by the same line are significantly different ($p<0.05$).
Any two means underscored by the same line are not significantly different.

Experiment 2

Body weight differed between mice fed 0.05% Na^+ or 3.00% Na^+ for 13 days (15.47g ± 0.91 versus 13.48g ± 1.24, respectively, $p< 0.05$). NE concentration did not differ between treatments in either the heart or kidney. However, fractional and total NE turnover rates significantly differed between treatments and were 57% and 79% lower, respectively, in mice fed 0.05% diet compared to their high Na^+ fed counterparts (Table III-4, Figure III-8).

Experiment 3

Body weight did not differ between mice fed the 0.05% Na^+ and 3.00% Na^+ diets for 30 days, (18.1g ± 1.2 versus 17.6g ± 1.2, respectively). NE concentrations and NE turnover rate in brain, heart, kidney, and IBAT did not differ between treatments (Table III-5, Figure III-9).

TABLE III-4

Effects of 0.05% or 3.00% sodium intake for 13 days on tissue norepinephrine turnover: Exp 2

	Organ Weight (mg)	Endogenous NE (nmol/g)	Fractional NE Turnover (%/h)	Total NE Turnover (nmol/g/h)
KIDNEY				
0.05% Na^+	91.8 ± 0.7[1]	4.74 ± 0.16[2]	19.62 ± 1.27	0.852
3.00% Na^+	92.4 ± 1.0	5.05 ± 0.14	23.53 ± 1.19	1.119
HEART				
0.05% Na^+	74.9 ± 0.3	5.46 ± 0.26	17.06 ± 1.16	0.898
3.00% Na^+	73.8 ± 0.4	5.34 ± 0.10	27.56 ± 1.18*	1.607*

[1] Values given are means ± SEM for n=18 mice in each treatment group.
[2] n=5-6 mice per time point.

* different from mice fed 0.05% Na^+, $p<0.05$.

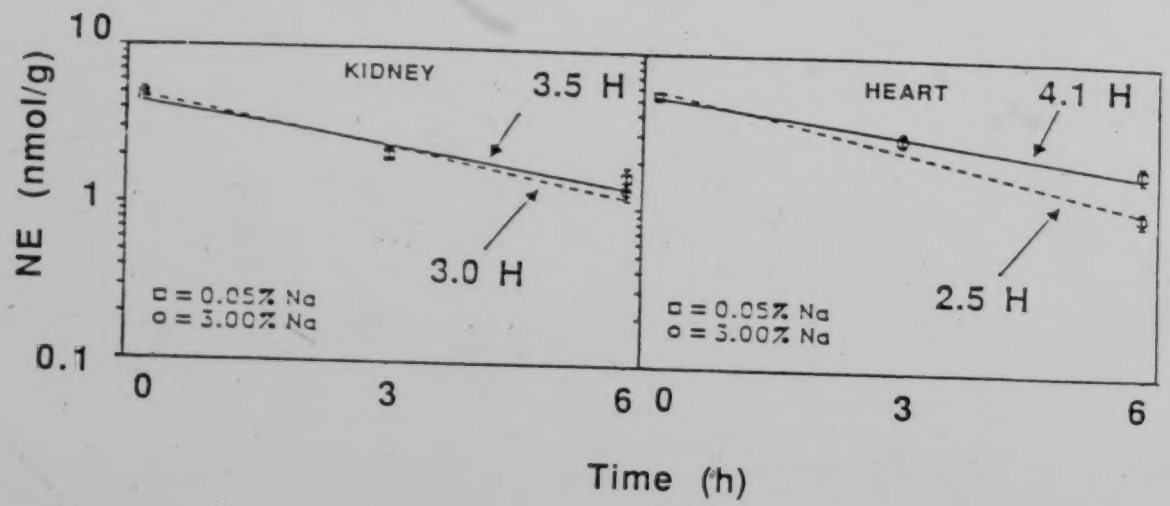

Figure III-8 Disappearance of NE from mouse kidney and heart after α-methyl-p-tyrosine administration in mice fed a 20% protein diet with 0.05% Na^+ (——) or 3.00% Na^+ (----) for 13 days (Exp 3). Each point represents mean ±SEM of 6 mice. Half-times for disappearance of NE in hours are shown for comparison purposes. In the heart, the slopes of the two lines are different ($p < 0.05$).

Equations for least squares fit of log transformed concentrations:
[y=log NE(pmol/g); x=time(h)]:

Kidney:	0.05% Na^+	y=3.638 - 0.085x	; r= -0.859
	3.00% Na^+	y=3.677 - 0.102x	; r= -0.912
Heart:	0.05% Na^+	y=3.721 - 0.074x	; r= -0.848
	3.00% Na^+	y=3.766 - 0.120x	; r= -0.930

TABLE III-5

Effects of 0.05 or 3.00% sodium intake for 30 days on tissue norepinephrine turnover: Exp 3

	Organ Weight (mg)	Endogenous NE (nmol/g)	Fractional NE Turnover (%/h)	Total NE Turnover (nmol/g/h)
BRAIN				
0.05% Na^+	207.1 ± 0.7 [1]	4.02 ± 0.08 [2]	11.48 ± 0.72	0.436
3.00% Na^+	199.4 ± 0.5	4.06 ± 0.13	11.66 ± 0.53	0.454
HEART				
0.05% Na^+	94.9 ± 0.3	5.06 ± 0.13	22.92 ± 1.39	1.028
3.00% Na^+	93.3 ± 0.4	5.21 ± 0.09	23.59 ± 1.10	1.173
KIDNEY				
0.05% Na^+	109.6 ± 0.3	4.73 ± 0.09	18.86 ± 1.67	0.748
3.00% Na^+	113.1 ± 0.6	4.96 ± 0.06	22.76 ± 1.48	0.927
IBAT				
0.05% Na^+	114.4 ± 1.5	4.23 ± 0.21	18.33 ± 1.88	0.564
3.00% Na^+	109.2 ± 1.0	4.33 ± 0.26	22.64 ± 1.62	0.776

[1] Values are means ± SEM for n=22-24 mice in each treatment group.
[2] n=7-8 mice per time point.

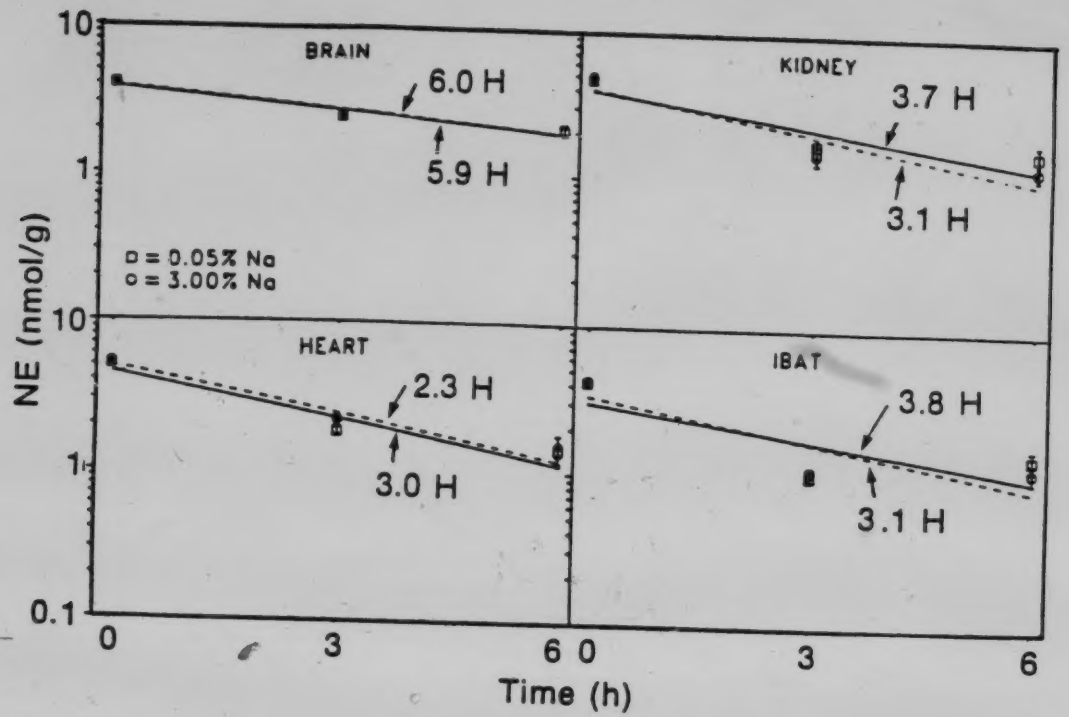

Figure III-9 Disappearance of NE from mouse brain, heart, kidney, and interscapular brown adipose tissue (IBAT) after α-methyl-p-tyrosine administration in mice fed a 20% purified protein diet with 0.05% Na^+ (——) or 3.00% Na^+ (----) for 30 days (Exp 3). Each point represents mean ±SEM of 8 mice. Slopes of the two lines within each treatment are not different ($p>0.05$). The numbers shown are half-times for disappearance of NE in hours.

Equations for least squares fit of log transformed concentrations:
[y=log NE(pmol/g); x=time(h)]:

Brain:	0.05% Na^+	y=3.580 - 0.050x	; r= -0.836
	3.00% Na^+	y=3.590 - 0.051x	; r= -0.900
Heart:	0.05% Na^+	y=3.652 - 0.099x	; r= -0.842
	3.00% Na^+	y=3.697 - 0.102x	; r= -0.897
Kidney:	0.05% Na^+	y=3.598 - 0.082x	; r= -0.730
	3.00% Na^+	y=3.610 - 0.099x	; r= -0.824
IBAT:	0.05% Na^+	y=3.488 - 0.080x	; r= -0.679
	3.00% Na^+	y=3.535 - 0.098x	; r= 0.791

D. Discussion

Free NE excretion declined temporally in response to prolonged consumption of a low (0.05%) Na^+ diet, apparent by 13 days after the initiation of the low Na^+ diet, but did not change over the same time period in normotensive mice consuming a 3.00% Na^+ diet. NE turnover rate in the heart only was significantly lower with similar treatment duration in mice fed the low Na^+ compared to the high Na^+ diet and thus may be responsible for the observed decline in free NE excretion associated with consumption of the 0.05% Na^+ diet. Chronic (30 day) consumption of the 0.05% Na^+ diet did not affect central or peripheral NE turnover, indicating a time-dependent relation between Na^+ intake and sympathetic activity assessed by free or total NE excretion and cardiac NE turnover.

The results of the present study are significant for several reasons. First, by studying the temporal adaptation pattern of fluid balance and Na^+, creatinine, free NE and DA excretion to altered Na^+ intake within a physiological range, the previously undetermined time-dependent relation between Na^+ intake, Na^+ excretion, and sympathetic activity was assessed. Second, this is the first report investigating the time course of the effect of altered Na^+ intake on NE turnover in peripherally innervated organs of normotensive animals. The series of experiments are unique by virtue that they quantify 'normal' physiological changes in sympathetic activity of normotensive animals in response to altered Na^+ intake within a physiological range. As such, experimental findings are distinct from those previously reported with normotensive animals investigating the effects of extreme, chronic Na^+ supplementation (Kaufman and Vollmer 1984; Dietz et al. 1980; Saavedra et al. 1983). Furthermore, observations allow direct comparisons between NE turnover and catecholamine excretion methodologies for SNS assessment.

Acute 1 or 5 day increases in Na^+ intake from 0.05% through 0.10% to 3.00% Na^+ have been shown not to affect NE turnover in brain, heart, kidney or IBAT in normotensive mice (Simon et al. 1987). Present findings extend these observations to show that sympathetic activity, assessed by NE excretion and cardiac NE turnover rate,

declines transiently in response to low (0.05%) Na^+ feeding for 13 to at least 18 days. Results indicated that 24h urinary free NE excretion was a reliable indicator of SNS activity in that it paralleled changes in cardiac NE turnover in response to feeding the low Na^+ diet. These observed differences in free NE excretion and cardiac NE turnover rate between mice fed low and high Na^+ diets support the hypothesis that the level of activity of the sympathetic nervous system is modified in response to alterations in dietary Na^+ intake. These results confirm that a positive relationship exists between Na^+ intake and noradrenergic activity and are in acord with previous studies on the effect of Na^+ loading in normotensive humans (Nicholls et al. 1980; Dustan 1985; Berglund 1982; Parfrey et al. 1981; Campese 1982).

Results suggest that differences in NE excretion and NE turnover rate produced by alterations in dietary Na^+ reflect suppression of sympathetic activity in response to prolonged consumption of a low Na^+ diet rather than a stimulatory effect of Na^+ on sympathetic activity as reported in studies with normotensive humans (Nicholls et al.1980; Parfrey et al. 1981; Dustan 1985) or hypertensive animals (Campese 1982; Parfrey et al. 1981). Previous normotensive human studies analyzing plasma and urinary NE responses to alteration in Na^+ intake have based stimulatory effects of high Na^+ intake on noradrenergic activity relative to that seen with consumption of a low Na^+ diet. Similarly, studies of hypertensive animals have reported that increases in dietary Na^+ enhance sympathetic activity by a one-time-point, direct comparison to those levels of activity seen with lower Na^+ intake, used as controls. Comparison of fractional or total NE turnover rates across different experiments (Chapter II and Chapter III), support that cardiac NE turnover is lower in mice fed diets containing 0.05% Na^+, subchronically. Present findings stress the importance of conducting temporal studies and analyzing sympathetic responses over time, from onset to termination of treatment, for accurate data interpretation.

The findings clearly indicate that a time-dependent relationship exists between Na^+ intake and sympathetic activity. Significant differences in urinary free NE and DA levels

occurred between days in response to low and high Na^+ feeding. Free NE excretion declined over time in response to 0.05% Na^+ feeding such that differences between treatments became consistent by day 6, 13 days after initiation of the low Na^+ feeding regime. However, the inhibitory effect of low Na^+ consumption on sympathetic activity was transient since NE turnover in mice chronically (30 day) fed the 0.05% or 3.00% Na^+ diets did not differ in brain or any organ examined. Significant day-by-treatment interactions occurred in relation to urinary free NE and DA levels, further supporting time dependency of sympathetic response to Na^+ intake.

Dietary Na^+ exerted an effect on free NE excretion such that differences in free NE values between mice fed 0.05% or 3.00% Na^+ were consistently significantly different by the sixth treatment day. Decreases in free NE excretion in response to feeding a 0.05% Na^+ diet for 13 days appeared to be a result of decreased NE turnover rate in the heart, reflecting decreased NE release and presumably NE production in accordance with steady-state kinetics (Elser 1984). Thus baroreceptor-mediated sympathetic control of cardiovascular function may be affected by Na^+ intake, the results therefore supporting an important role for cardiac sympathetic activity in circulatory compensations associated with altered dietary sodium. Statistically, it appears unlikely that sympathetic regulation of renal hemodynamics is important in the maintenence of Na^+ and fluid homeostasis in response to physiological alterations in Na^+ intake. However, a nonsignificant lowering effect of the 0.05% Na^+ diet on renal fractional and total NE turnover was apparent, indicating a possible relation between sympathetic activity and Na^+ conservation within the kidney with consumption of a low Na^+ diet.

The results concur with findings of Meldrum et al. (1985) wherein noradrenergic activity, assessed by portal vein and anterior hypothalamus NE concentrations, decreased in response to low (0.05%) Na^+ feeding for 7 to 9 days in spontaneously hypertensive rats and normotensive controls. Gradin, Dahlof and Persson (1986) similarly reported that a

low dietary sodium intake of 0.02% Na^+ reduces neuronal NE release in spontaneously hypertensive rats when compared to those fed normal (0.57%) or high (5.22%) Na^+ diets.

The direct relationship seen between Na^+ intake, urinary Na^+, and DA excretion is in accord with previous findings (Alexander et al. 1974, Carey et al. 1981; Imbs et al. 1984; Morgunov and Baines 1981; Alexander et al. 1974; Oates et al. 1979). Observed antinatriuresis in response to low Na^+ diet as reflected by low Na^+ excretion and urine volume, is attributed to decreased vasodilation of renal vasculature, increased filtration fraction and Na^+ reabsorption secondary to decreased renal dopaminergic activity (Morgunov and Baines 1981; Alexander et al. 1974; Oates et al. 1979).

The direct correlation seen between Na^+ intake and free NE excretion is unusual and does not support the antinatriuretic role for NE suggested by Morgunov and Baines (1981) and Oates et al. (1979). The significance of altered NE excretion observed in relation to Na^+ homeostasis is difficult to determine as alpha and beta adrenergic receptors in renal juxtaglomerular cells have been reported to exert opposite influences on renin release (Zanchetti 1985). Furthermore, although evidence indicates that the catecholamines NE and DA regulate Na^+ excretion, Na^+, in turn may regulate catecholamine release and excretion as indicated by studies showing Na^+ intake can markedly affect the function of both the central and peripheral nervous systems by altering nerve activity and/or the storage and release of neurotransmitter (Chalmers 1975; de Champlain et al. 1968; Giachette et al. 1979). Therefore, the mechanism and physiological significance underlying the direct relationship between Na^+ intake and urinary NE excretion observed remains unclear. It is possible the antinatriuretic response produce by decreased renal dopaminergic activity, as reflected by DA excretion, was inappropriate and counter balanced to some extent by decreased noradrenergic activity to maintain Na^+ homeostasis. However, since Na^+ excretion was observed to decline in response to low Na^+ feeding, it is apparent that changes in Na^+ balance are predominantly attributed to the changes in dopaminergic activity associated with antinatriuresis.

Differences in urinary creatinine values may reflect more efficient recovery of creatinine from mice fed the high (3.00%) Na^+ diet due to their greater urine volume and urine flow, improving collection from the metabolism cages compared to mice fed the low Na^+ diet. The small urine volumes relative to water intake suggest this possibility. Alternatively, the higher creatinine excretion may be attributable to increased renal filtration secondary to blood volume expansion as result of Na^+ loading (Laederach and Weidmann 1987). The close association between Na^+ intake and Na^+ excretion within treatments indicates the urine collections were complete and that differences between water intake and urine volume may merely reflect loss due to evaporation. Therefore, the possibility remains that the dietary treatment did affect creatinine excretion and that creatinine is not truely a physiological covariate under these circumstances.

No effect on central or peripheral NE turnover was observed with chronic, 30 day 0.05% or 3.00% Na^+ feeding. The findings are in accord with recent research of Dawson and Oparil (1987) reporting no difference in renal NE concentration between normotensive Sprague Dawley rats fed diets containing either 'normal' or high (3.4%) Na^+ concentrations for three weeks, although NE concentation alone is a poor and inconsistent indicator of noradrenergic activity.

NE concentration declined monoexponentially in all tissues examined except IBAT demonstrating that one of the major assumptions of steady-state kinetics had been met. The decline in NE concentration in IBAT apperared to be biphasic and may underestimate NE turnover rate. These results are similar to those reported in a previous study where the fit of the data for the monoexponential curve was less significant in IBAT than in other organs studied when NE concentration was determined at seven time points over a 6 hour period (Johnston and Balachandran 1987).

Our results suggest that the low-Na^+-induced reduction in sympathetic activity, assessed through urinary NE excretion and cardiac NE turnover, is transient and that noradrenergic homeostasis can be regained over time. Molecular studies investigating

adrenergic receptor binding properties or ion exchange across neuronal membranes are needed to determine the underlying mechanism responsible for observed changes in sympathetic activity.

The importance of altered sympathetic activity in the pathogenesis of hypertension associated with a high Na^+ intake is questionable in view of the present findings. Our findings confirm a direct relationship exists between dietary Na^+ and SNS activity. However, differences in noradrenergic activity previously attributed to increased Na^+ intake appear to be a result of a decline in sympathetic activity in response to low Na^+ feeding rather than a stimulatory effect of Na^+ on SNS activity. The effects of Na^+ within a physiological range on SNS activity in normotensive mice appear to be transient.

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IV. Appendices

Appendix 1

Assay of Tissue Norepinephrine for Turnover Determination

1. Previously weighed frozen tissues were homogenized in 1 ml 0.4M perchloric acid ($HClO_4$), 20 µl 0.2M ethylene diaminetetraacetic acid (EDTA), 10 µl 1.0M sodium bisulfite ($NaHSO_3$) and 100 µl 7x 10-6M 3,4- dihydroxybenzamine (DHBA) as an internal standard.

2. Homogenates were centrifuged at 9000 rpm for 10 min. at -10°C and aliquots of the supernatants transferred to 5 ml vials containing 35 mg acid-washed alumina and 2 ml Tris buffer (pH 8.6).

3. Samples were agitated in a mehanical shaker for 15 min and supernatants removed by aspiration.

4. Alumina was washed twice with HPLC -grade water. NE and DHBA were eluted from the alumina with 200 µl 0.2M $HClO_4$.

5. Eluates were injected onto the high performance liquid chromatography system.

Appendix 2

Assay of Urinary Sodium

1. 100 µl urine aliquots were diluted to 100 ml in volumetric flasks and aspirated into a SP9 Phillips atomic absorption spectrophotometer set to the flame emission (FES) mode.

2. SP9 operating parameters used for sodium, determination:

Fuel	Acetylene	
Support Gas	Air	
Acetylene Pressure	4.5-5.5	psi
Air pressure	10-20	psi
Wavelength	589	nm
Wavelength scan	3	nm/min
Band Pass	0.5	nm
Burner Height	17	mm
Damping	0.1	s

3. Analytical parameters used for sample determination and standard calibration using SP9 computer

Time	0.2	s
Number	2	
Program	4	

4. Standards used for calibration and sodium determination

Standard	Sodium Concentration mmol/litre	FES
1	0.024	20.9
2	0.048	37.7
3	0.120	64.7
4	0.240	96.3

Appendix 3

Assay of Urinary Creatinine

1. 0.3 ml aliquots of urine were reacted with 3.0 ml alkaline picrate solution and 0.1 ml acid reagent provided by Sigma (Kit #555, Sigma Chemical Co. St. Louis, MO).

2. Quantification was based upon the formation of a red tautomeric form of creatinine utilizing the Jaffe reaction and measured by a spectrophometer set at 500nm.

Appendix 4

Analysis of Urinary Norepinephrine and Dopamine

Determination of Free Norepinephrine and Dopamine

1. 2ml aliquots of urine in 8 ml phosphate buffer (ph 7), 20 µl 0.1M sodium bisulfite ($NaHSO_3$), 40 µl 0.3M ethylenediaminetetraacetic acid (EDTA) and 200 µl dihyhydroxybenzamine (DHBA) as internal standard were applied to a miniature cation exchange isolation column
2. Columns were washed with 10 ml high performance liquid chromotography (HPLC) grade water (H_2O)
3. Columns were acidified with 1.5 ml 0.7M H_2SO_4 to desorb catecholamines from exchange resin.
4. Catecholamines were eluted from columns with 4.0 ml 2M ammonium sulfate [$(NH_4)_2SO_4$)] and collected in 5.0 ml vials.
5. 100 µl 4% thioglycolic acid, 50 mg acid-washed alumina and 2ml 2M Tris buffer (pH 8.6) were sequentially added to sample-containing vials.
6. Samples were agitated by a mechanical shaker for 10 minutes and supernatants removed by aspiration.
7. Catecholamines were eluted from the alumina with 500 µl 0.7M H_2SO_4 and vortexed for 10 seconds.
8. Eluates were injected onto the high performance liquid chromatography system.

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Education

1985-1988	University of Alberta. Candidate for MSc. in Nutrition Fall, 1988. Researching the mechanism by which dietary sodium induces hypertension.
1980-1985	University of Western Ontario. Completed Honors BSc. in Nutrition April 1985. Electives: Physiology and Anatomy.
1984-1988	University of Western Ontario. Candidate BSc. in Biology Summer, 1988.

Experience

1985-1987	*Graduate Teaching Assistant:* Department of Foods and Nutrition, University of Alberta. Position involved the organization and conduction of seminars dealing with biochemical aspects of various nutrients. Supervised by Dr. Tapan Basu.
October 1986	*Sessional Lecturer* : Department of Foods and Nutrition, University of Alberta. Course entailed laboratory biochemical assessment of body composition, to determine nutritional status.
Summer 1985	*Research Assistant* : Department of Anatomy, University of Western Ontario. Studied corneal epithelial migration during wound repair with electron microscopy. Supervised by Dr. R.C. Buck.
Summers 1982-1984	*Dietary Aide* : Calgary General Hospital. Supervised the production and administration of daily patient menus.
Summer 1984	*Receptionist* : Dr. John B. Simon Professional Corporation, Calgary. Part-time position.

Additional Experience

1986-1987

Elected *Vice President (Academic)* for the Graduate Students' Association, University of Alberta. Position involved the organization of the following:

- Annual Graduate Research Symposium
- Monthly Speaker Series
- Employment Forum

Served as an ex-officio member on University administrative committees including the Academic Development Committee (ADC) and the Negotiating Committee.

Elected graduate representative for the Faculty of Graduate Studies and Research (FGSR) Council and the FGSR Academic Appeals Committee (AAC).

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